

Supporting Information

Short Peptide Nucleic Acids Bind Strongly to Double Helical RNA

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Table of contents

Experimental Procedures	Pg. S2
UV melting curves of RNA and PNA triple helices	Pg. S4
Raw ITC data	Pg. S5
Analyzed ITC data	Pg. S28

Synthesis of PNA was done on Expedite 8909 synthesizer following the standard manufacturers protocol (2 μ mol scale) and using NovaSyn TG Sieber resin (Novabiochem) as a support, HATU as an activator and Fmoc-PNA-A(Bhoc)-OH, Fmoc-PNA-C(Bhoc)-OH, Fmoc-PNA-G(Bhoc)-OH and Fmoc-PNA-OH as monomers (purchased from Link Technologies Ltd, UK). Modification of PNA with L-lysine residue was done either manually (30 min coupling) or automatically on Expedite 8909 (using standard PNA coupling protocol) using Fmoc-L-lys(Boc)-OH and HATU. Cleavage from Sieber resin and global deprotection were done by treatment of PNA sequences on support with TFA/*m*-cresol (4/1, v/v) for 2.5 h. The crude PNA sample was precipitated from anhydrous ether. The solid was collected, dried, dissolved in HPLC grade water and purified by RP-HPLC on Xbridge Prep C-18 column (5 μ m, 10 mm \times 150 mm) at 60 °C eluting with a linear gradient 8%-13% of acetonitrile in water containing 0.1 % of TFA over 35 min, flow rate of 5 mL/min. Absorbency was monitored at 254 nm and 280 nm, and the fraction containing the major peak was collected, lyophilized to dryness to afford pure PNA samples. The molecular weight of the synthesized PNAs was confirmed by mass spectroscopy (MALDI TOF or LC/MS). The PNA was quantified following procedure described for DNA and RNA.¹

RNA was purchased from Dharmacon Inc and deprotected according to manufacturers recommendations. After deprotection RNA samples were purified using RP-HPLC on Xbridge Prep C-18 column (5 μ m, 10 mm \times 150 mm) at 60 °C eluting with a linear gradient (10.5%-22.5%) of mobile phase B in mobile phase A over 35 min, flow rate 5 ml/min. Mobile phase A was 0.1 M of triethylammonium acetate (pH = 7.0) in HPLC water and mobile phase B was a mixture of 0.1 M of triethylammonium acetate (pH = 7.0) in HPLC water and HPLC grade acetonitrile (60/40, v/v). Absorbency was monitored at a wavelength of 254 nm and 280 nm, and the fraction containing the major peak was collected, lyophilized to dryness to afford pure RNA samples. RNA was quantified using the extinction coefficient provided by Dharmacon.

¹ Puglisi, J. D.; Tinoco, I., Jr., Absorbance melting curves of RNA. *Methods Enzymol.* **1989**, *180*, 304-325.

ITC Experiments were done on a Nano ITC G2 (TA Instruments). RNA stock solution (35 μ L, 0.24 mM) was evaporated to dryness and the solid was dissolved in 1.6 mL of acetate buffer (100 mM of sodium acetate, 1.0 mM of EDTA, pH = 5.5). After degassing the RNA solution (0.95 mL, 0.00525 mM) was loaded into ITC reaction cell and the reference cell was loaded with degassed HPLC water. The instrument was equilibrated at 25 °C until the baseline was flat and stable. PNA stock solution (200 μ L, 0.24 mM) was evaporated to dryness and the solid was dissolved in 450 μ L of acetate buffer. After degassing the PNA solution (250 μ L, 0.096 mM) was loaded in titration syringe. The syringe was inserted into reaction cell and the instrument was equilibrated at 25 °C until the baseline was flat and stable. The stirring (250 rpm) was started and the instrument was equilibrated at 25 °C until the baseline was flat and stable. The following parameters were used:

- Experiment type: Incremental titration
- Stirring rate = 250 rpm
- Temperature set point = 25 °C
- Syringe size = 250 μ l
- Equilibration time = 300 sec
- Interval of individual injection = 260-480 sec
- Number of injections = 25-50
- Volume of individual injection = 5 μ l

The titration data (Figures S2-S24) were analyzed using NanoAnalyze software (TA Instruments) and independent model to obtain the fitting graph and thermodynamic data of the experiments (Table S1).

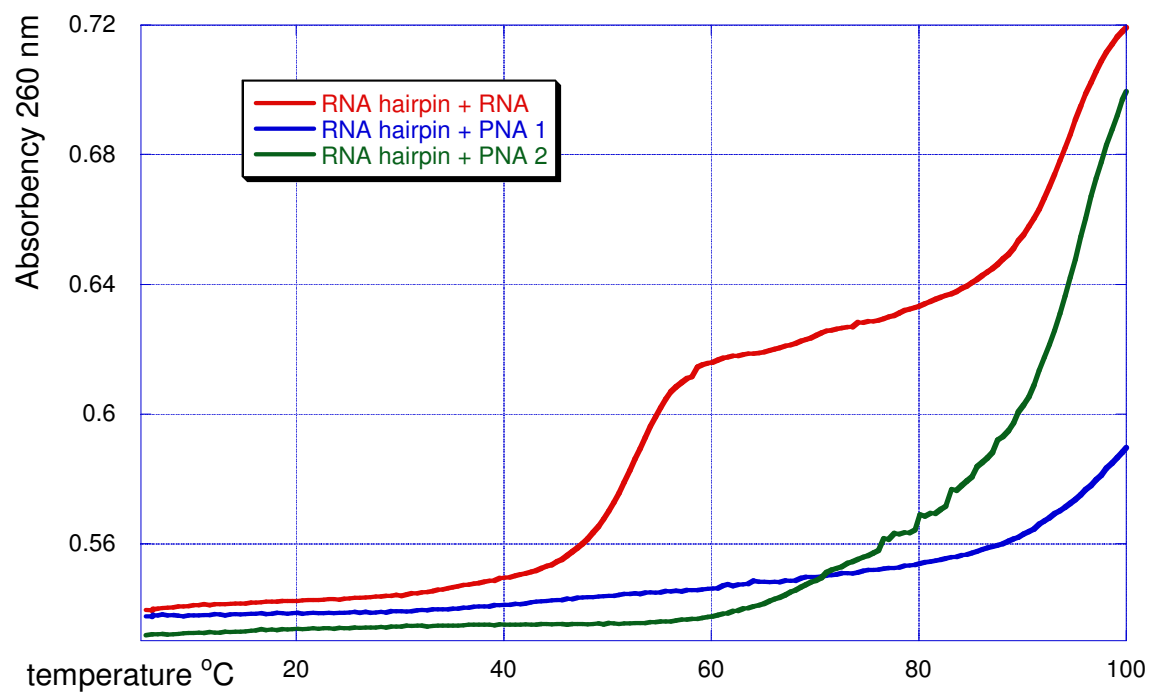


Figure S1. UV melting curves of triple helices formed by RNA hairpin (**HRP1**) and RNA and PNA third strands.

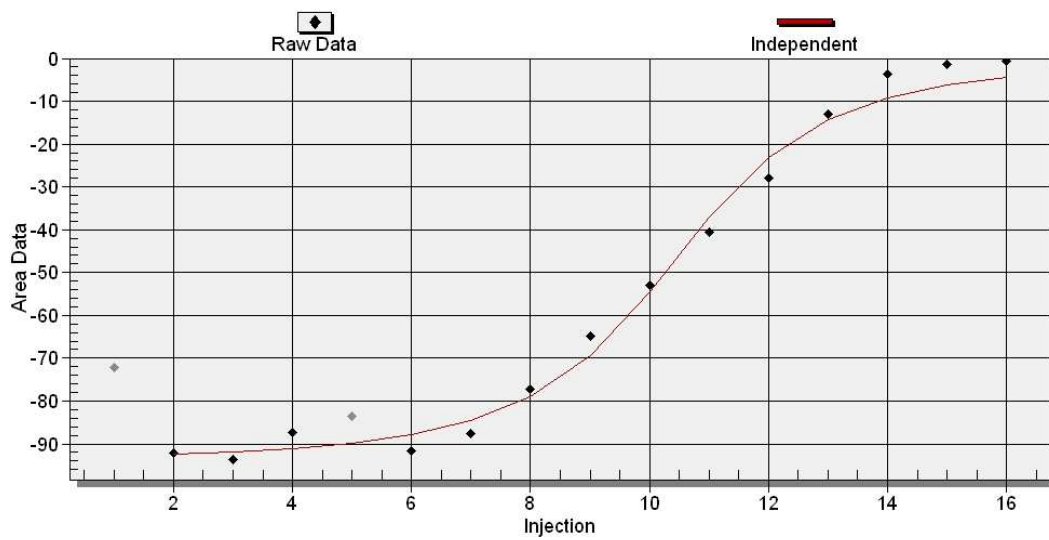
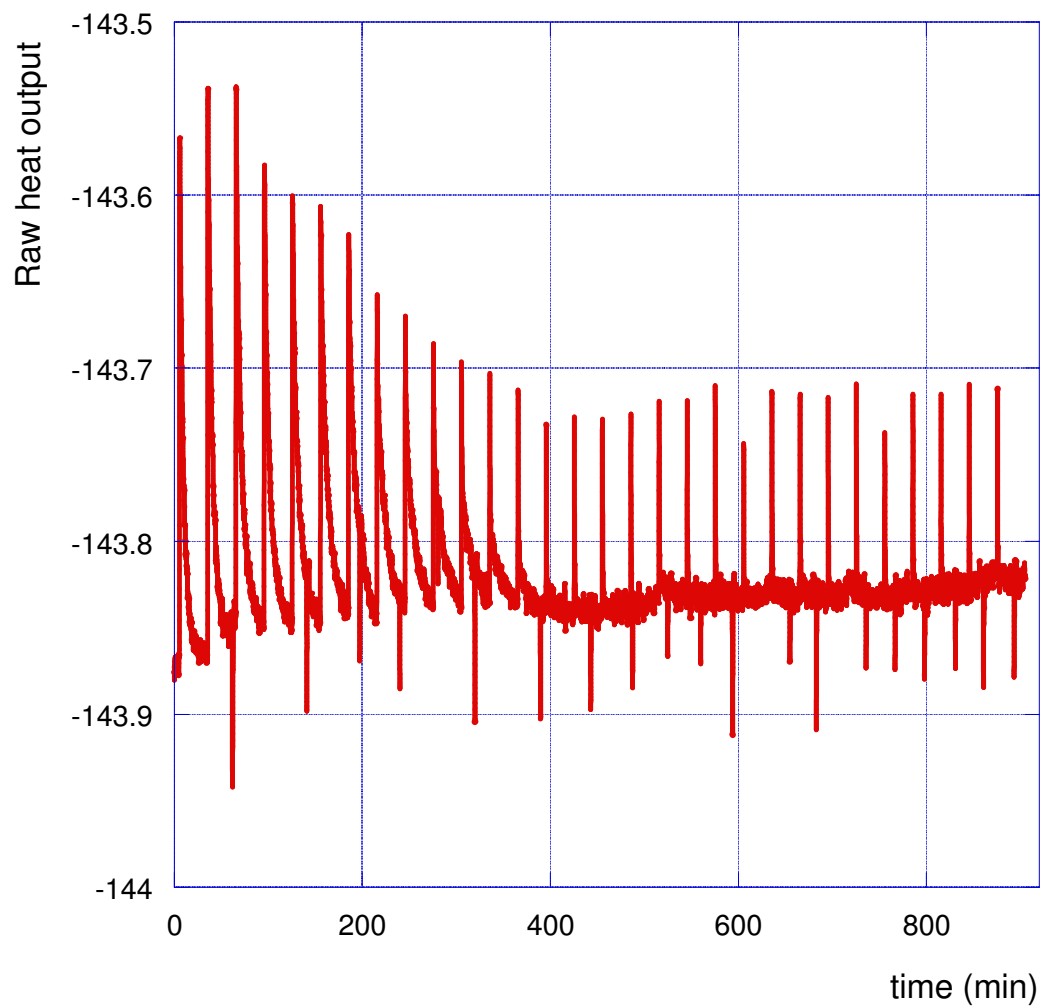


Figure S2. ITC data for binding of **RNA** to **HRP1**.

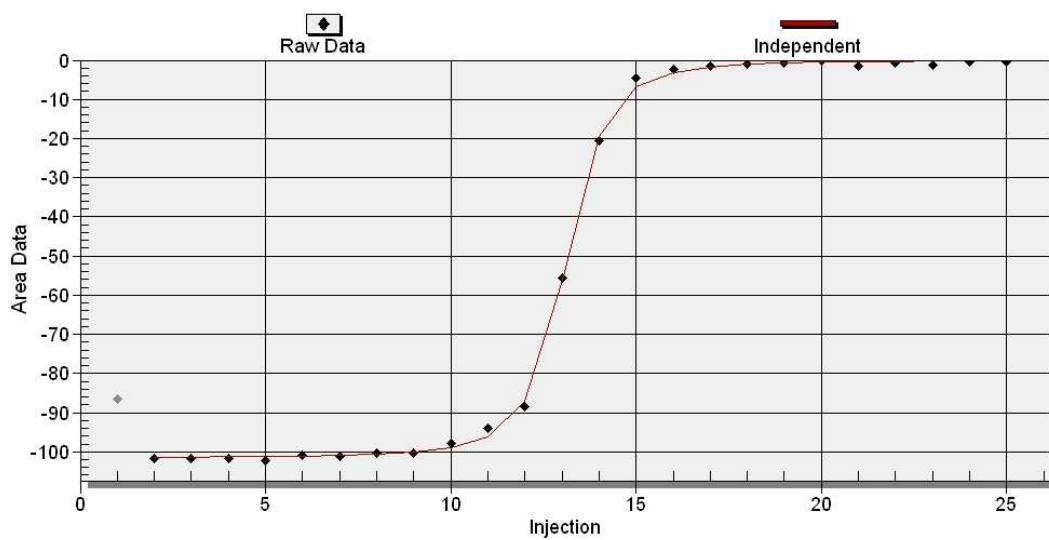
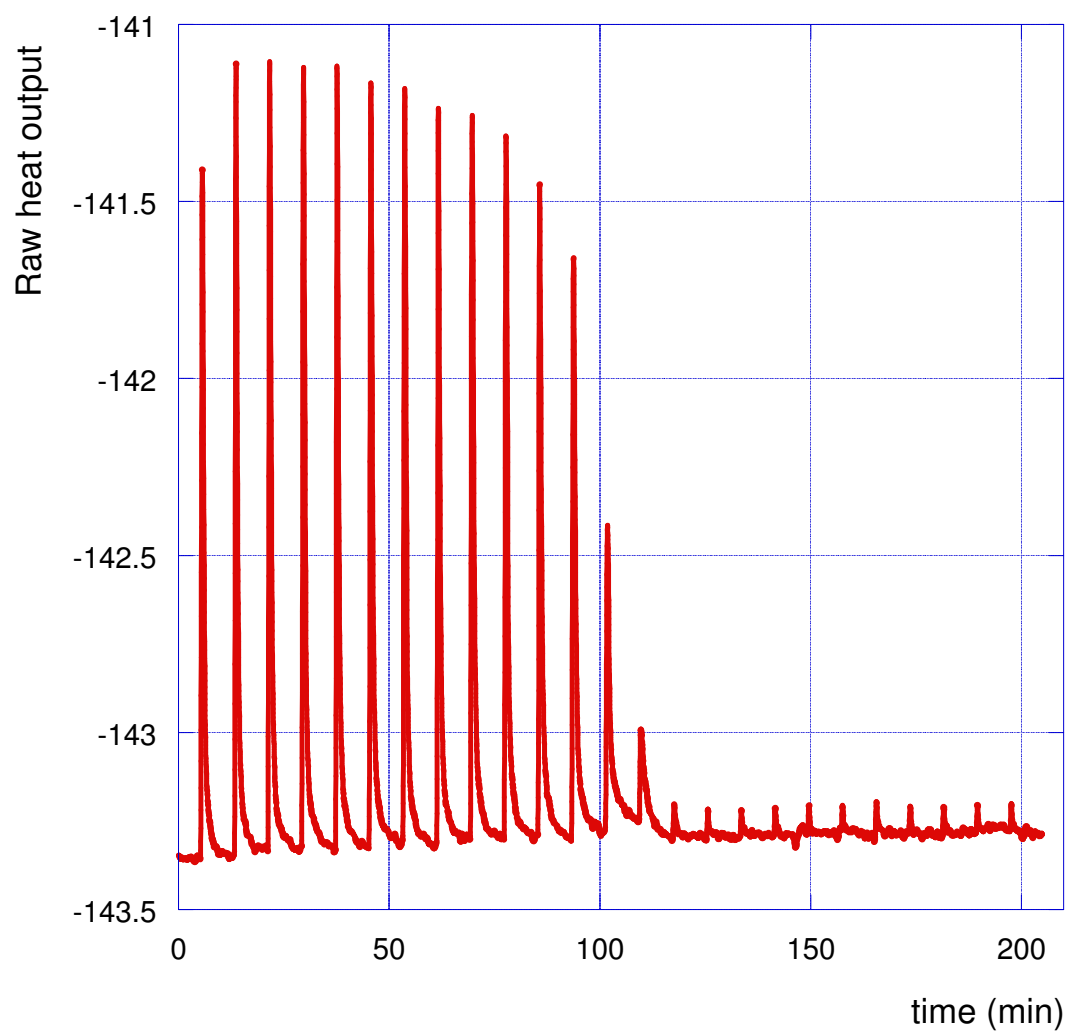


Figure S3. ITC data for binding of **PNA1** to **HRP1**.

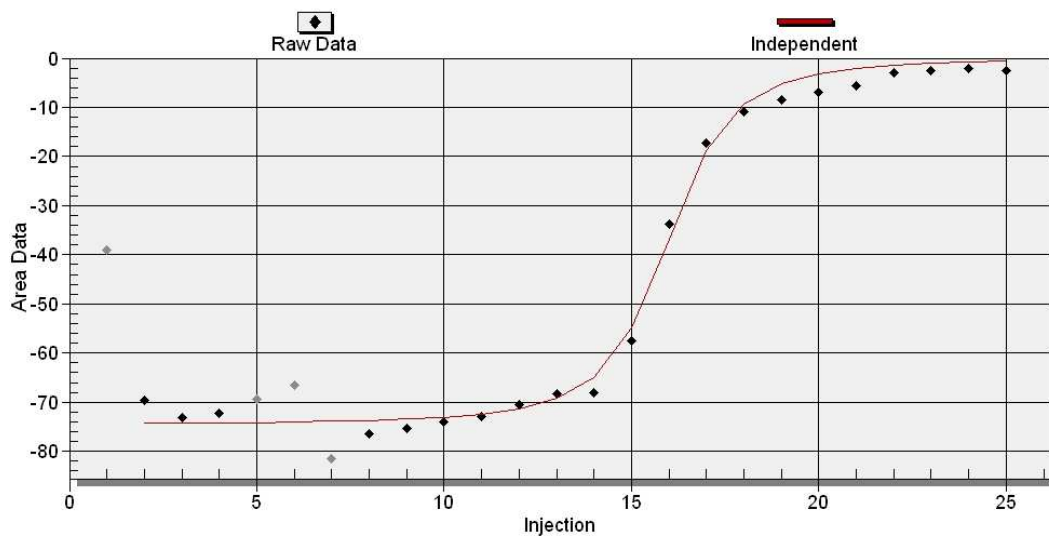
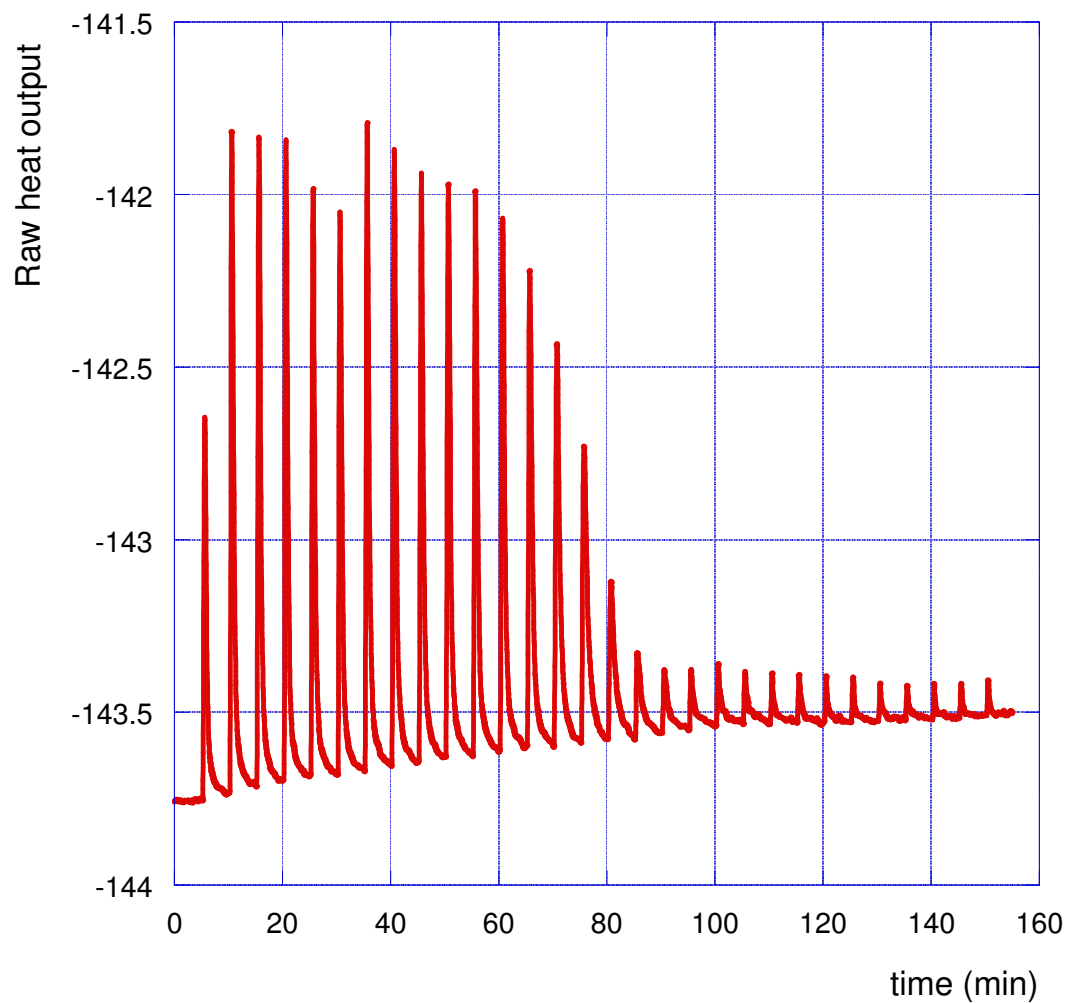


Figure S4. ITC data for binding of PNA2 to HRP1.

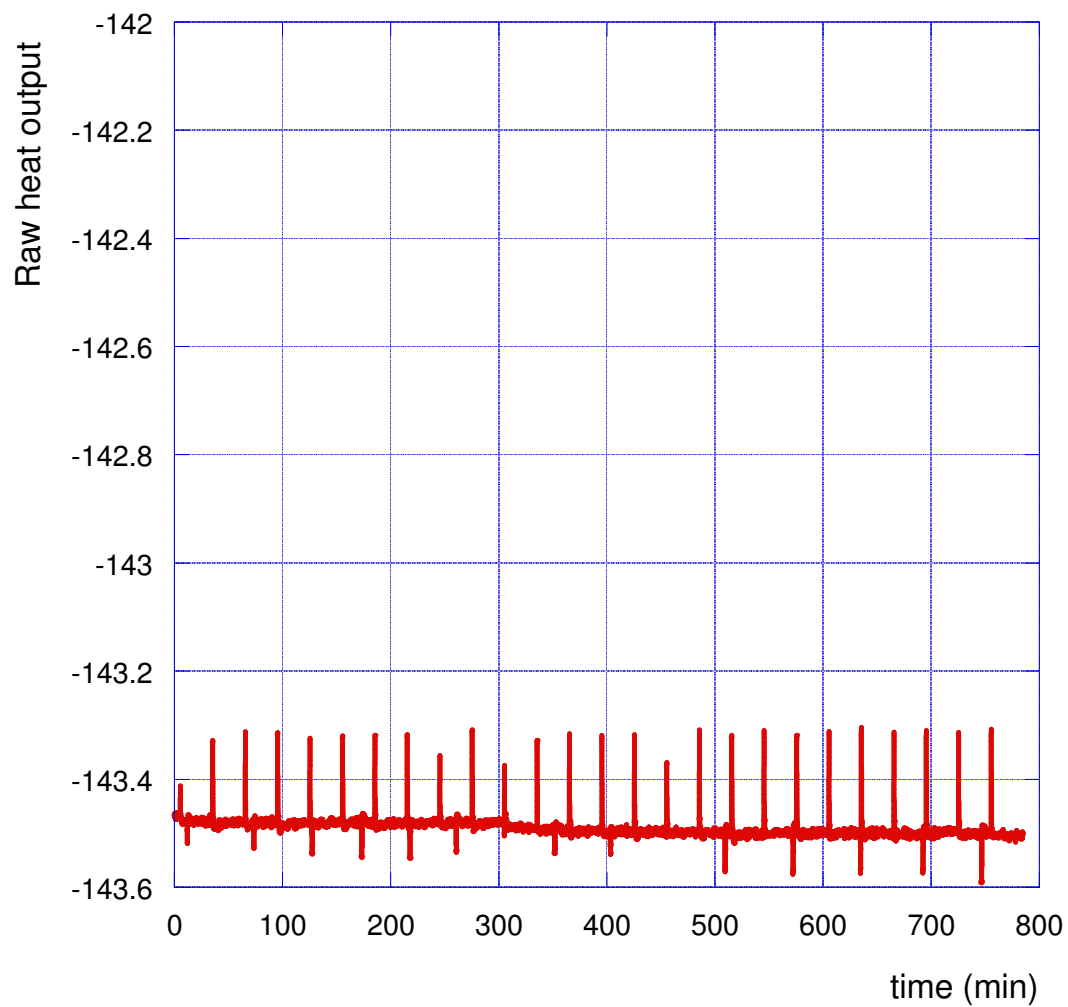


Figure S5. ITC data for binding of **DNA** to **HRP1**.

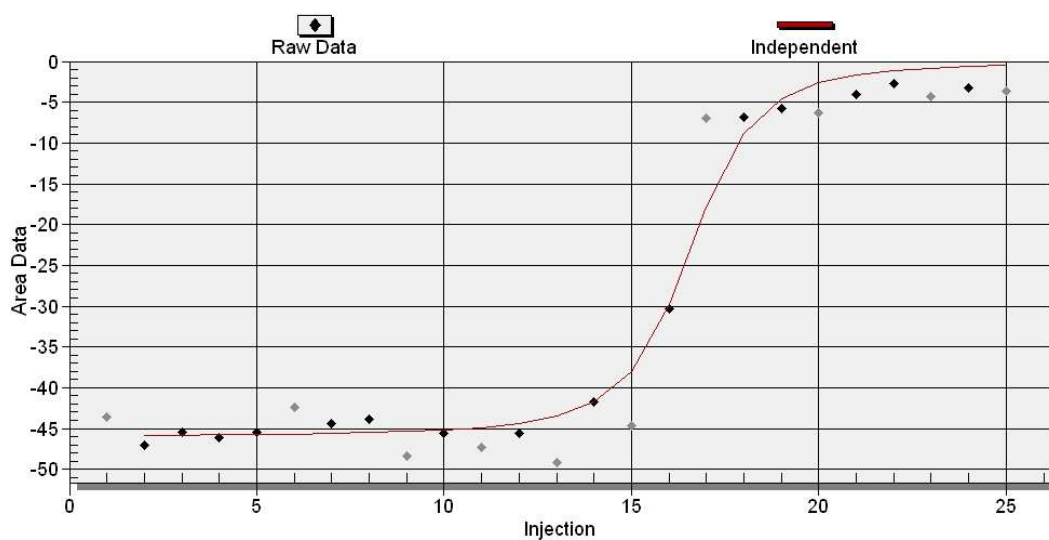
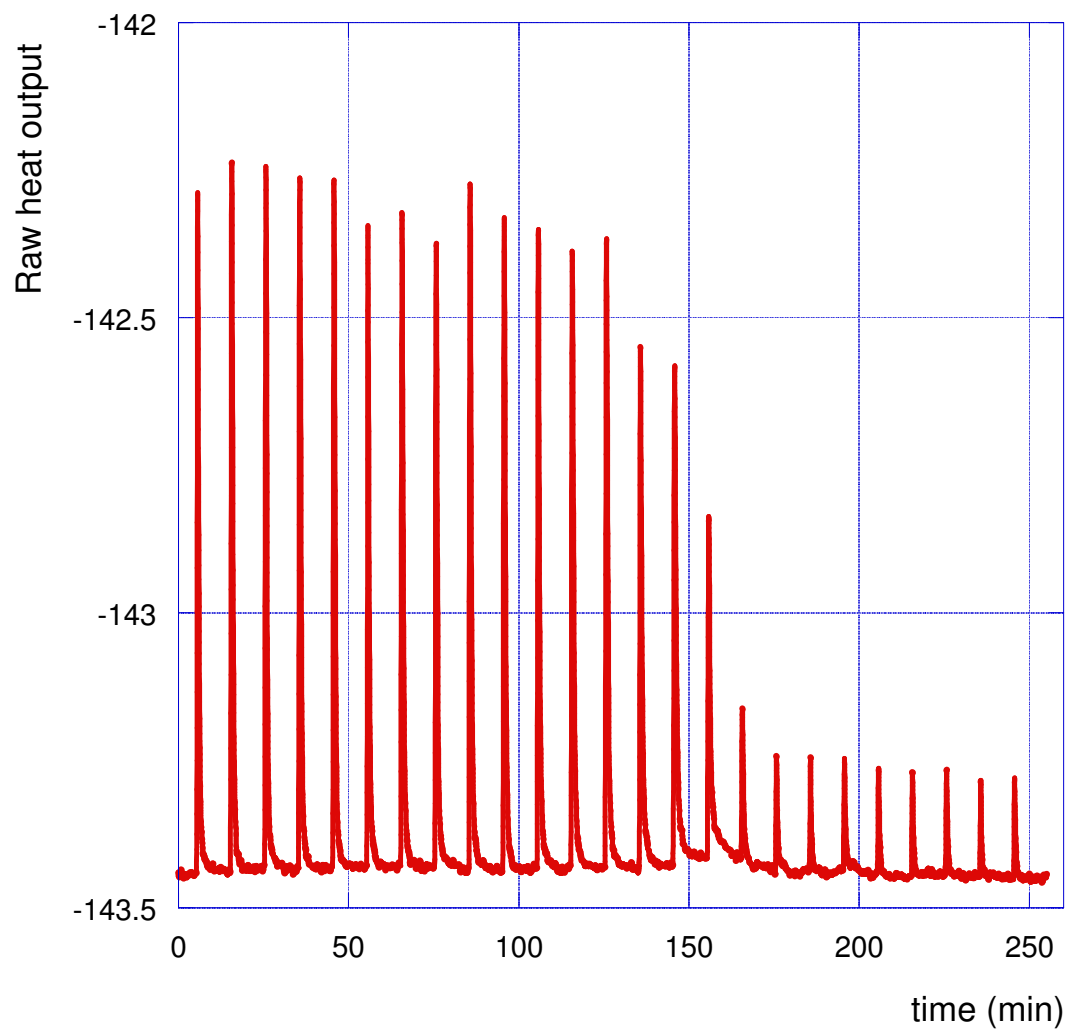


Figure S6. ITC data for binding of **PNA3** to **HRP1**.

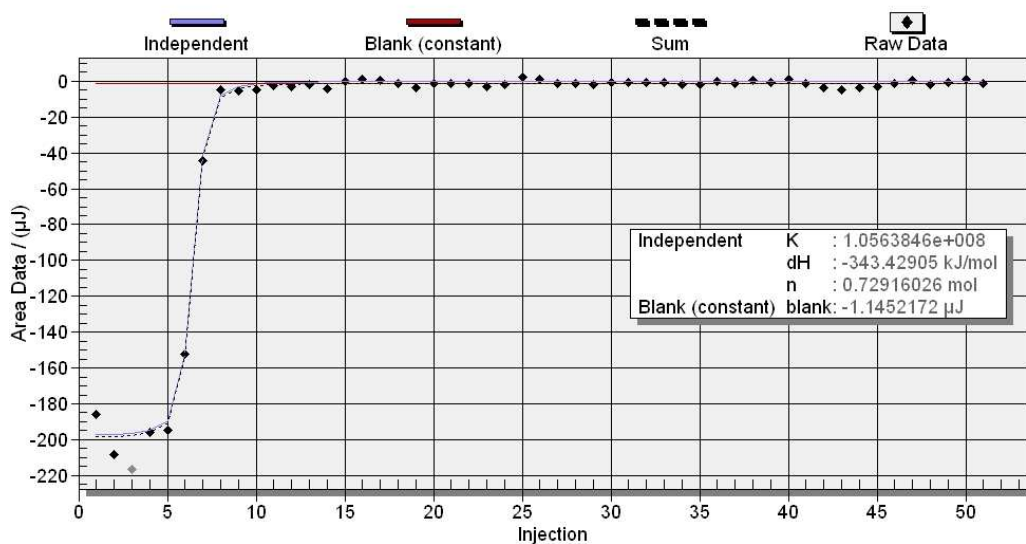
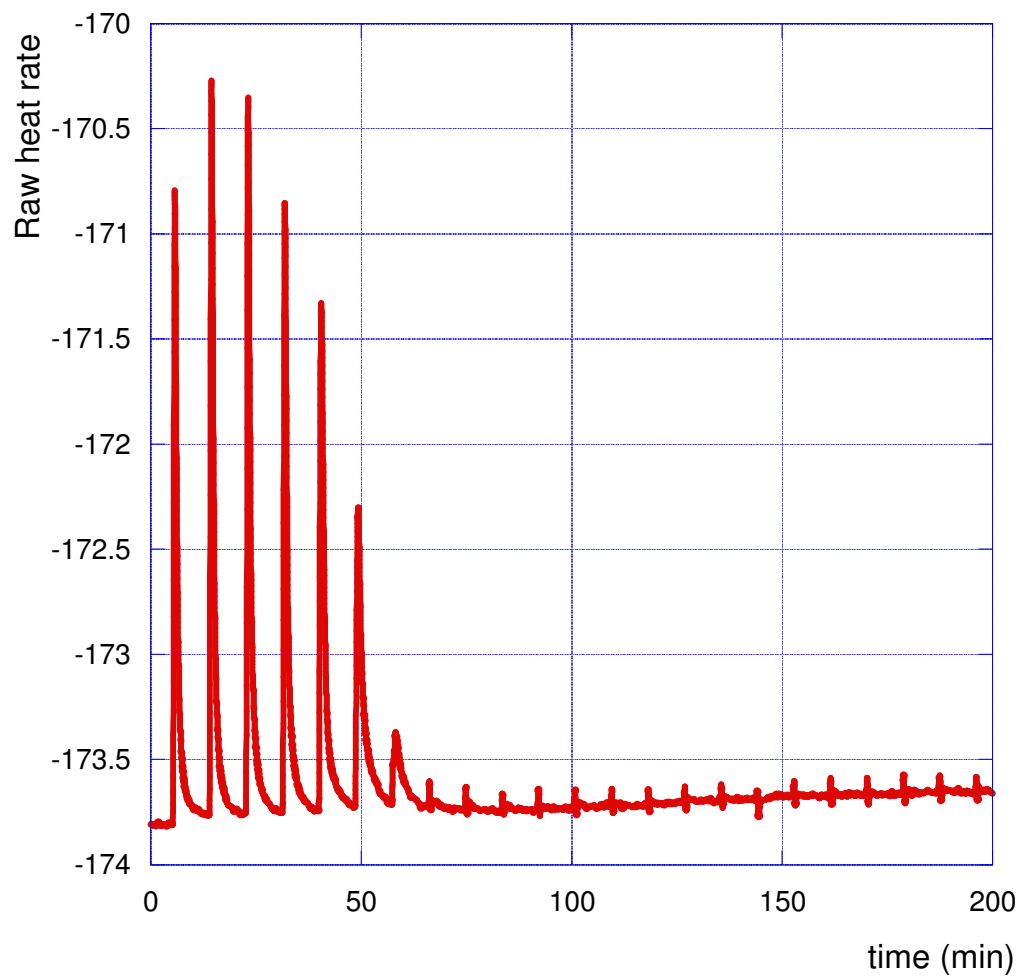
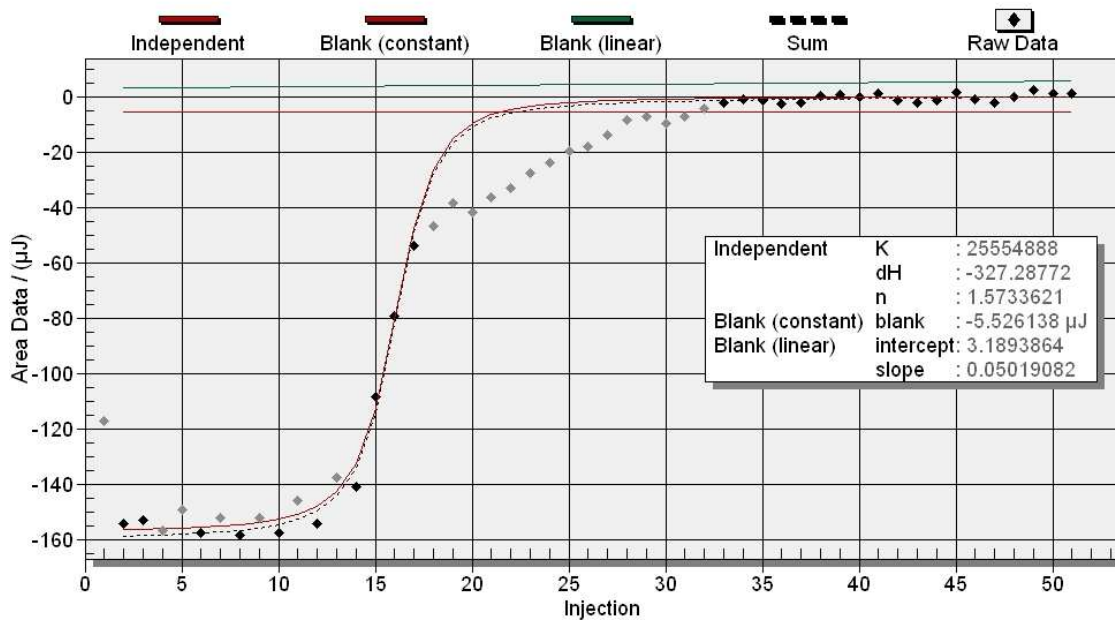


Figure S7. ITC data for reverse titration of **HRP1** (115 μM , 5mL injections) into **PNA1** (5.1 μM in calorimeter cell).

A. High affinity binding region.



B. Lower affinity binding region.

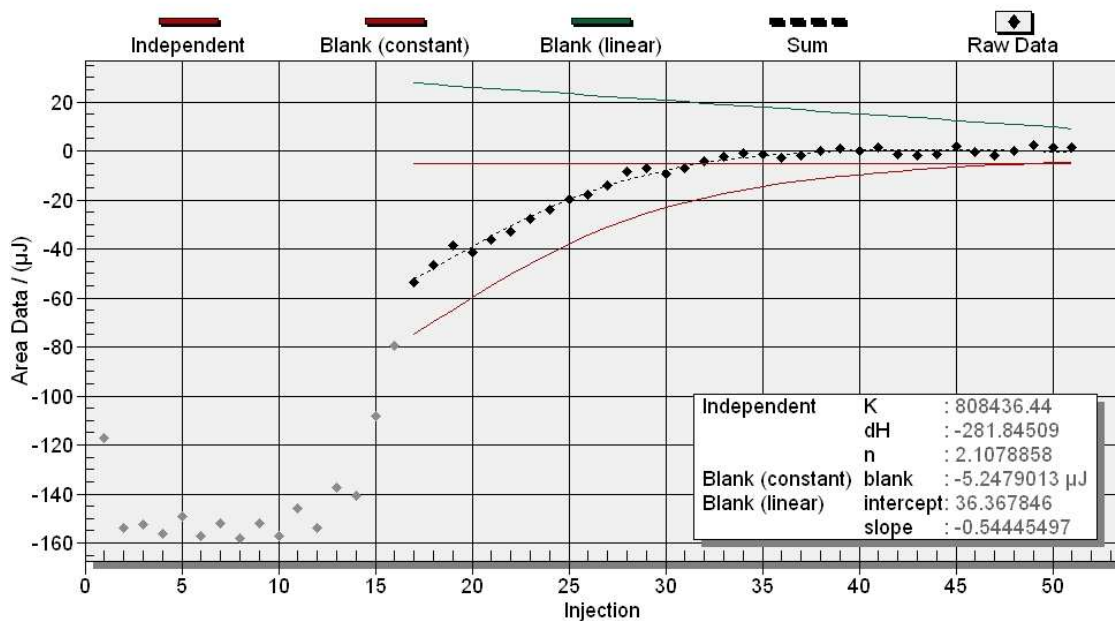


Figure S8. Fitting of ITC data for binding of PNA1 to DNA HRP5.

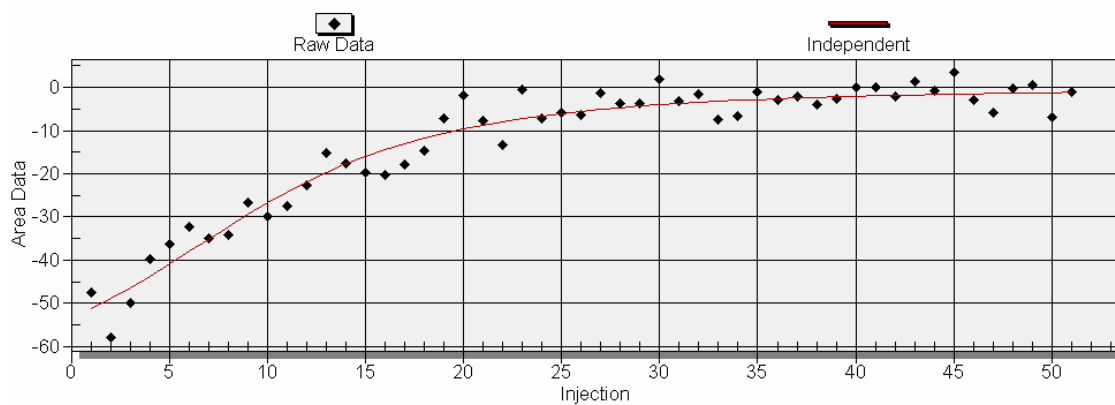
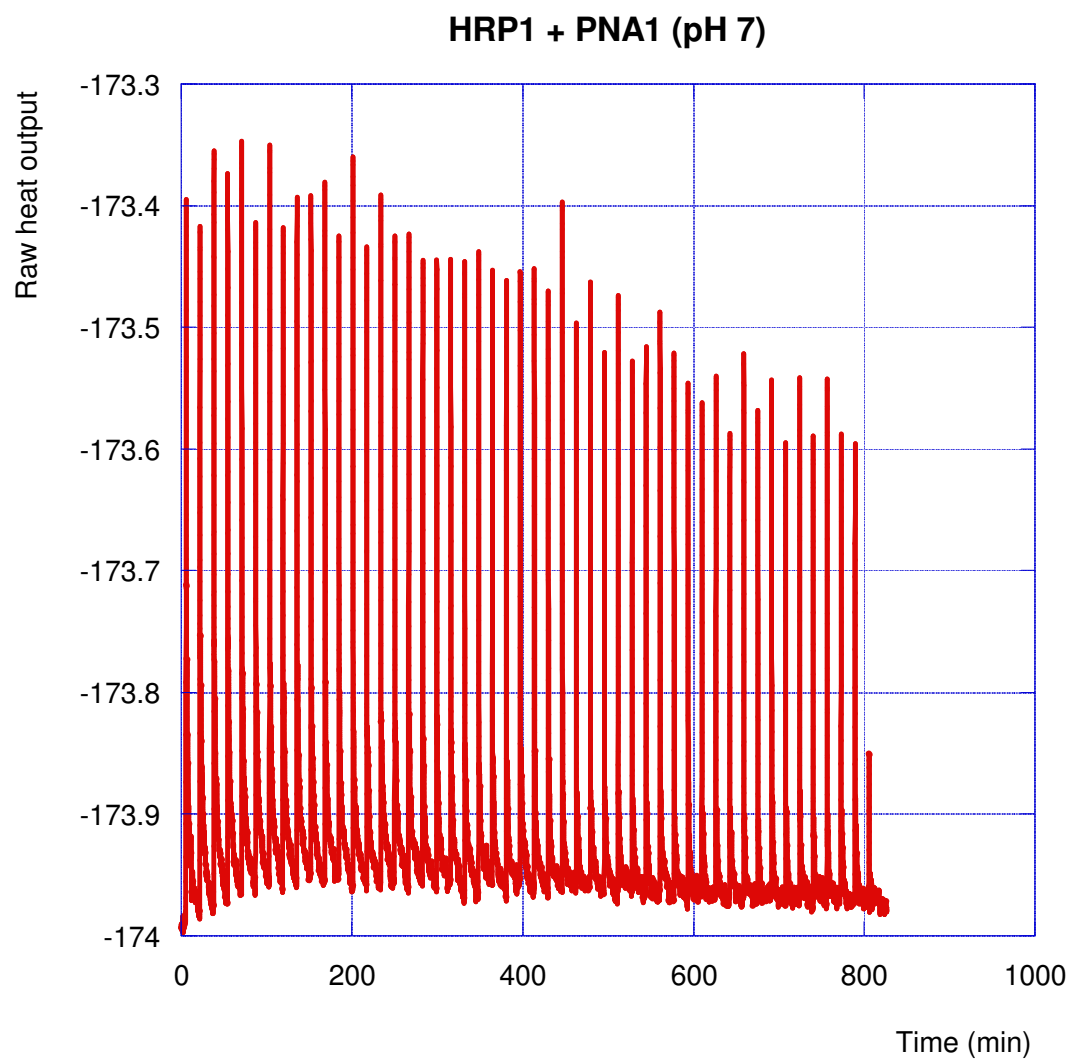


Figure S9. ITC data for binding of **PNA1** to **HRP1** (pH 7.0).

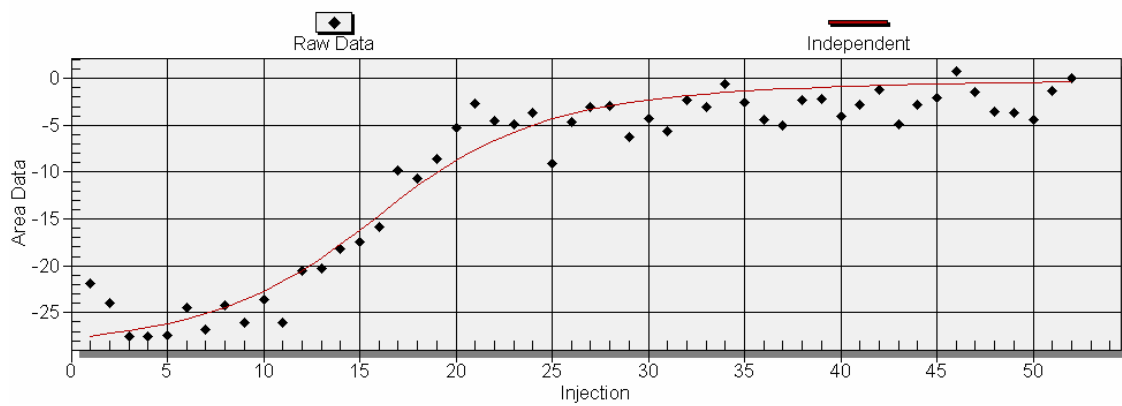
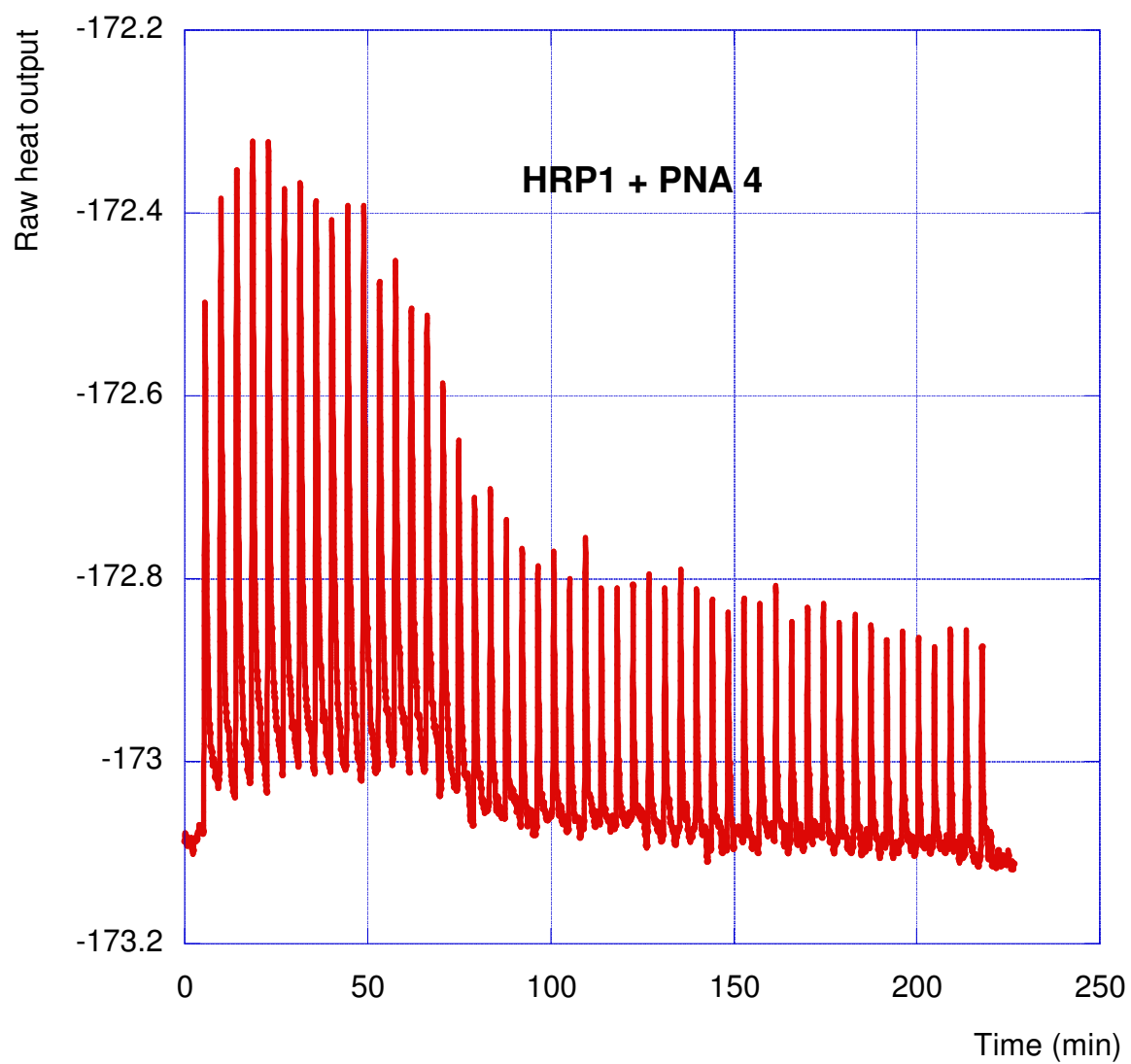


Figure S10. ITC data for binding of PNA4 to HRP1.

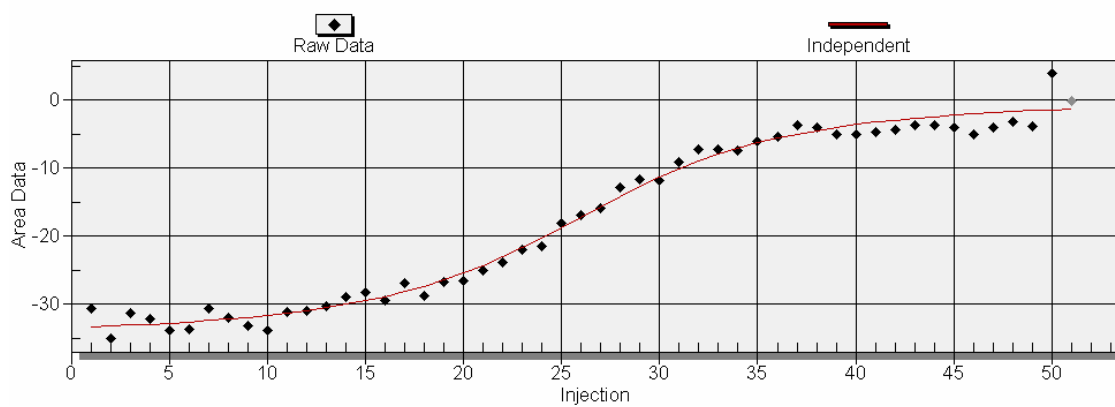
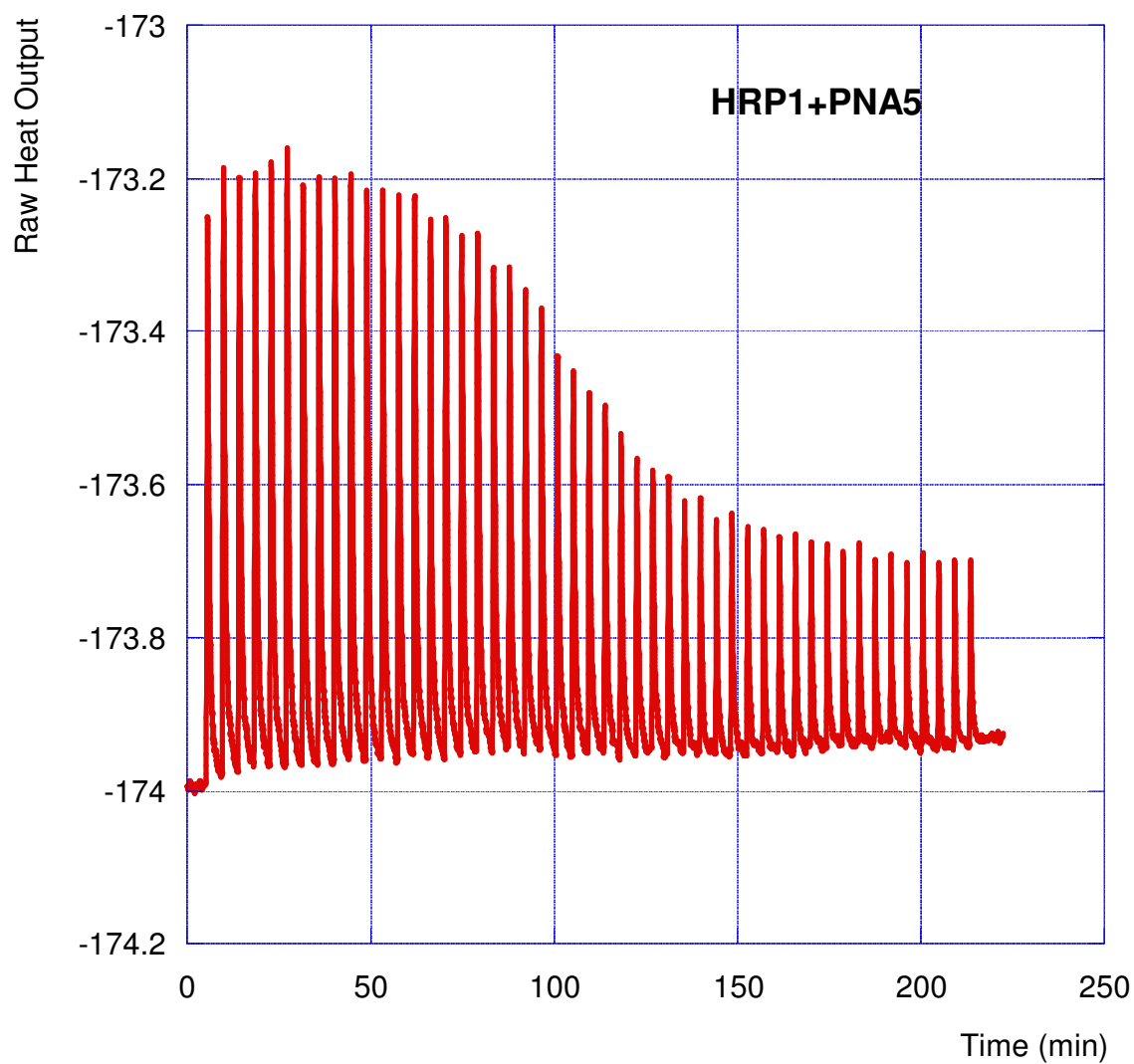


Figure S11. ITC data for binding of **PNA5** to **HRP1**.

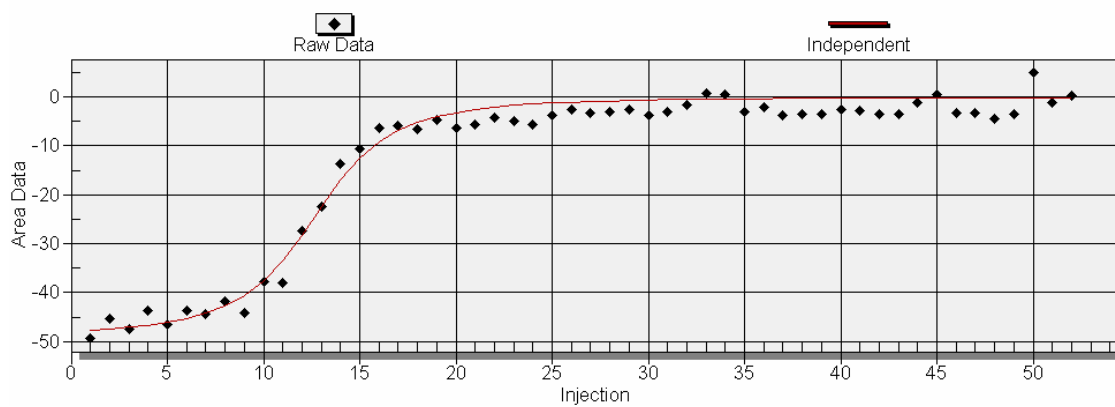
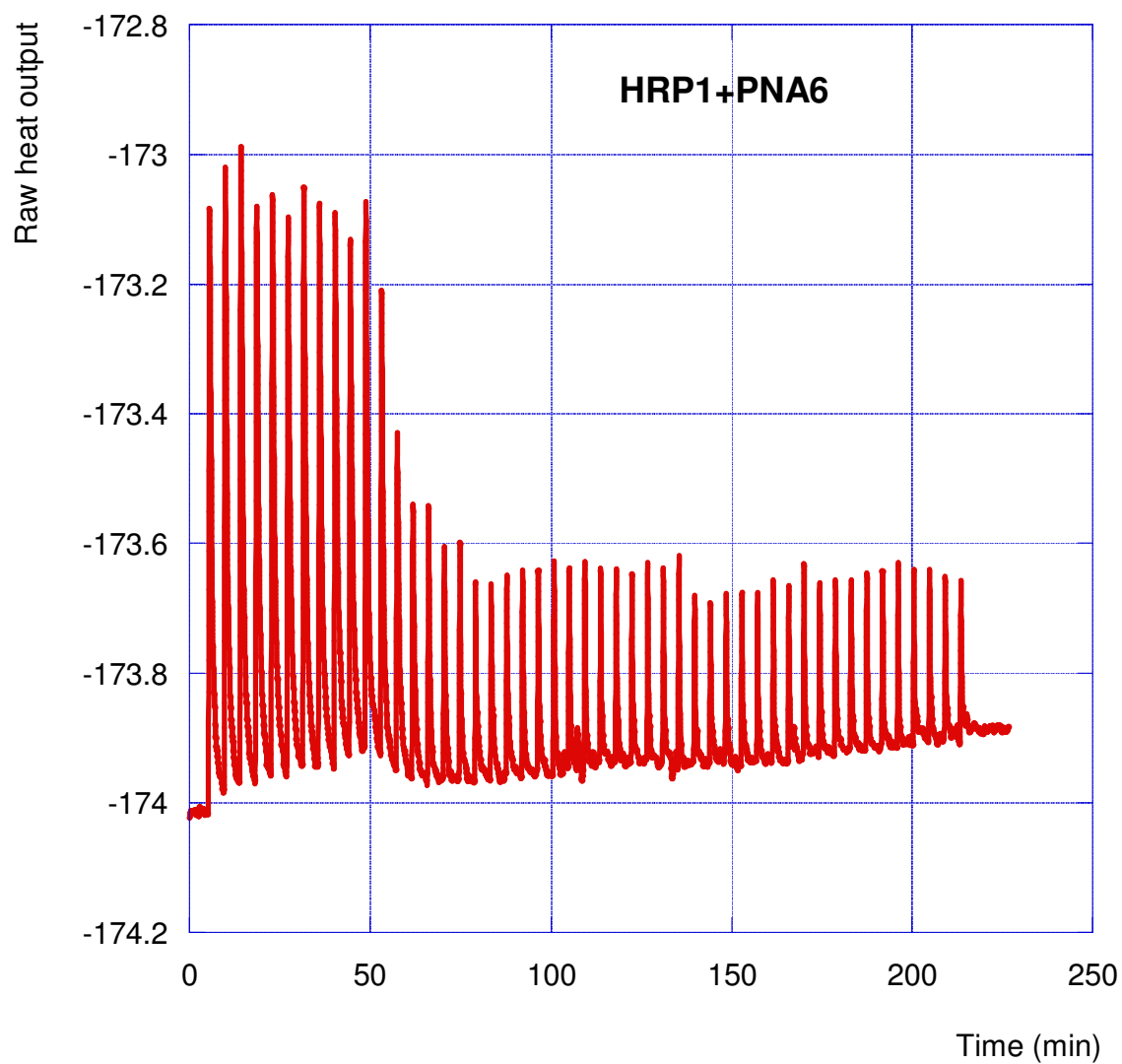


Figure S12. ITC data for binding of **PNA6** to **HRP1**.

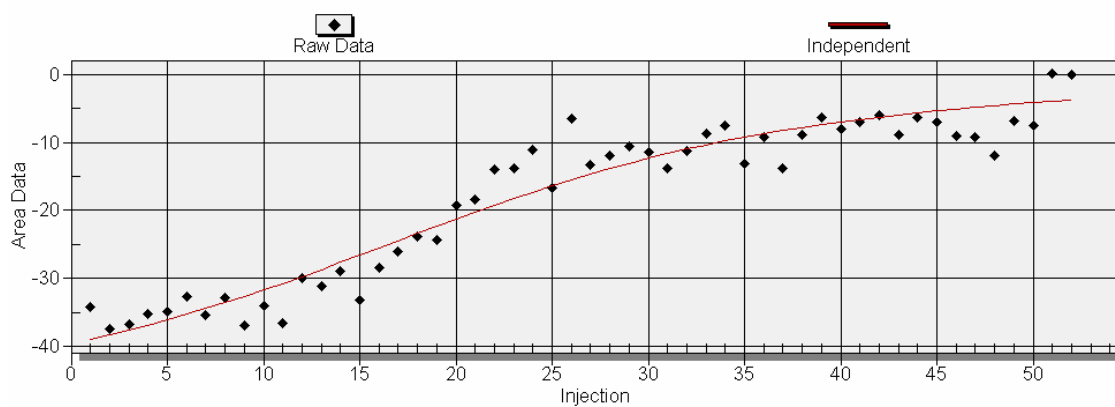
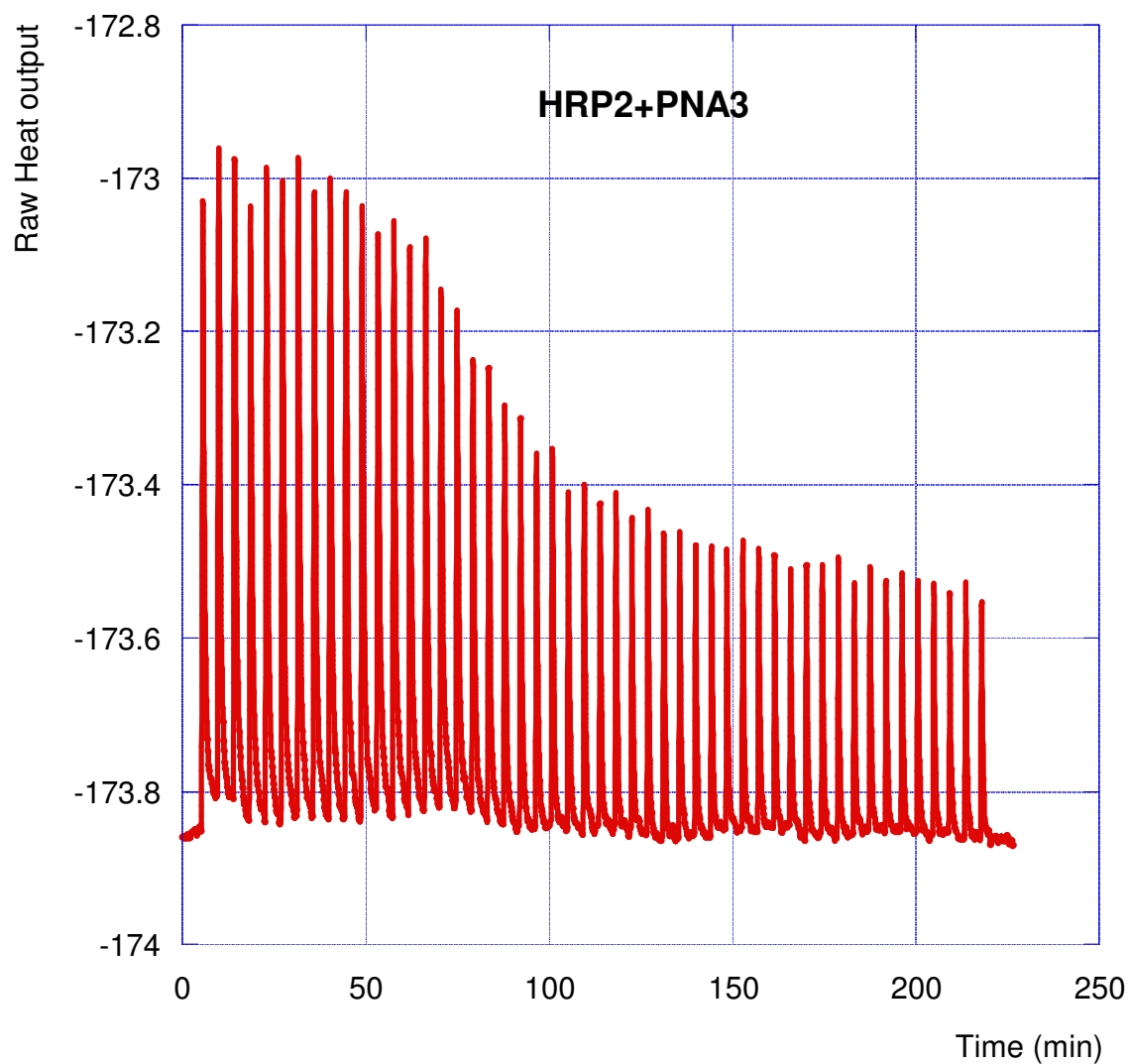


Figure S13. ITC data for binding of **PNA3** to **HRP2**.

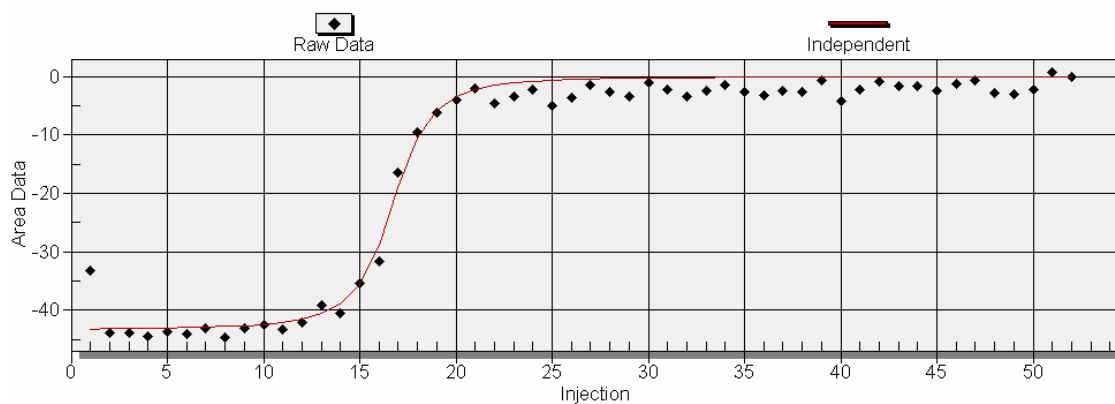
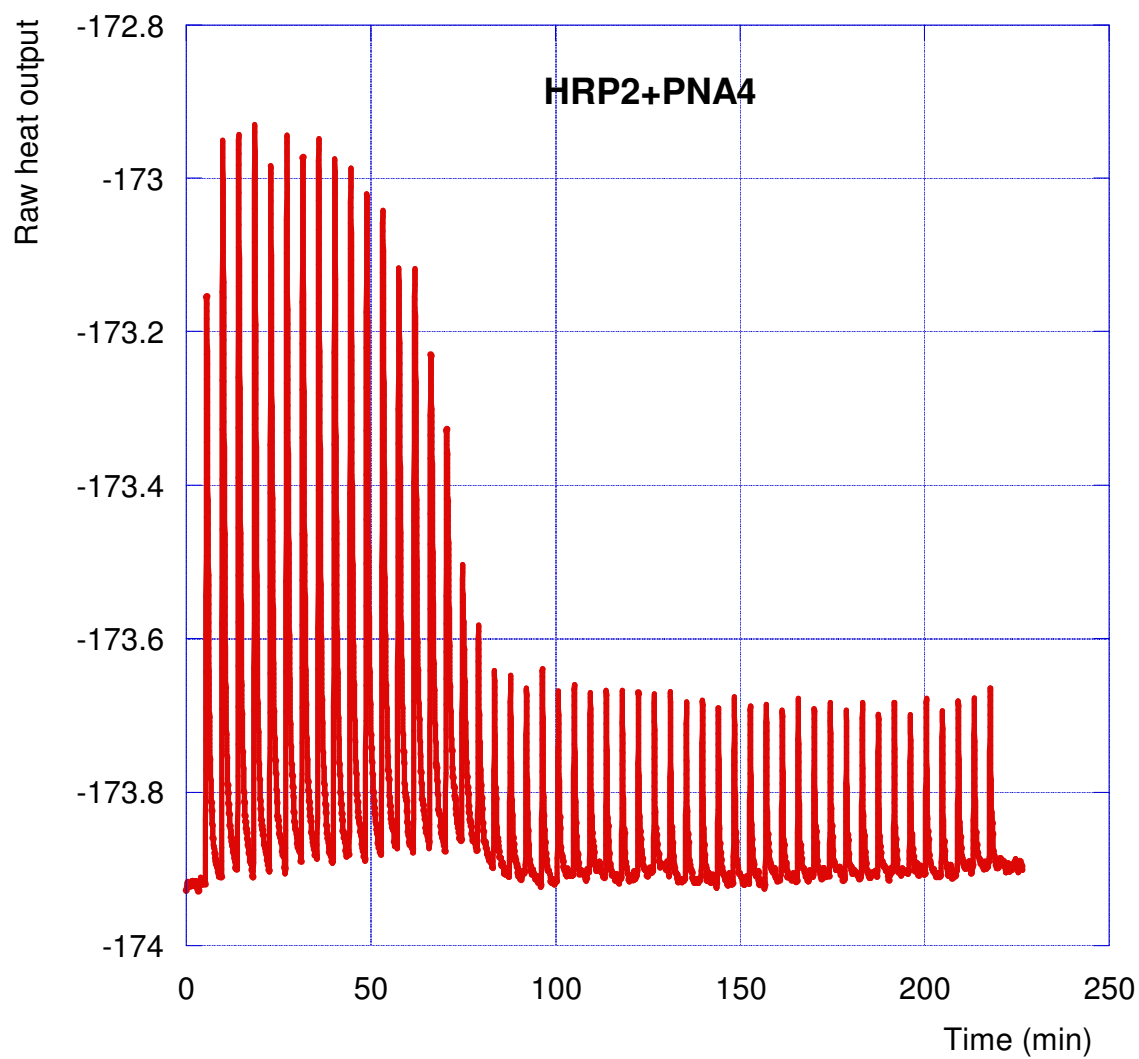


Figure S14. ITC data for binding of **PNA4** to **HRP2**.

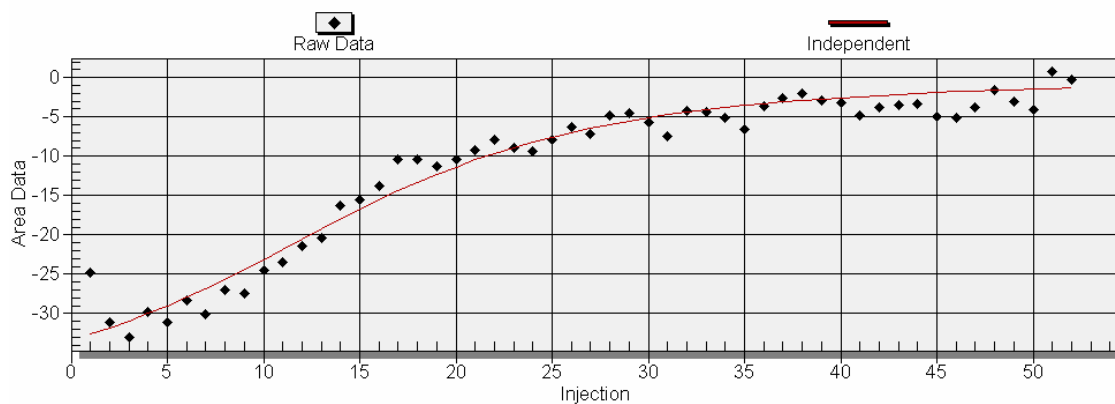
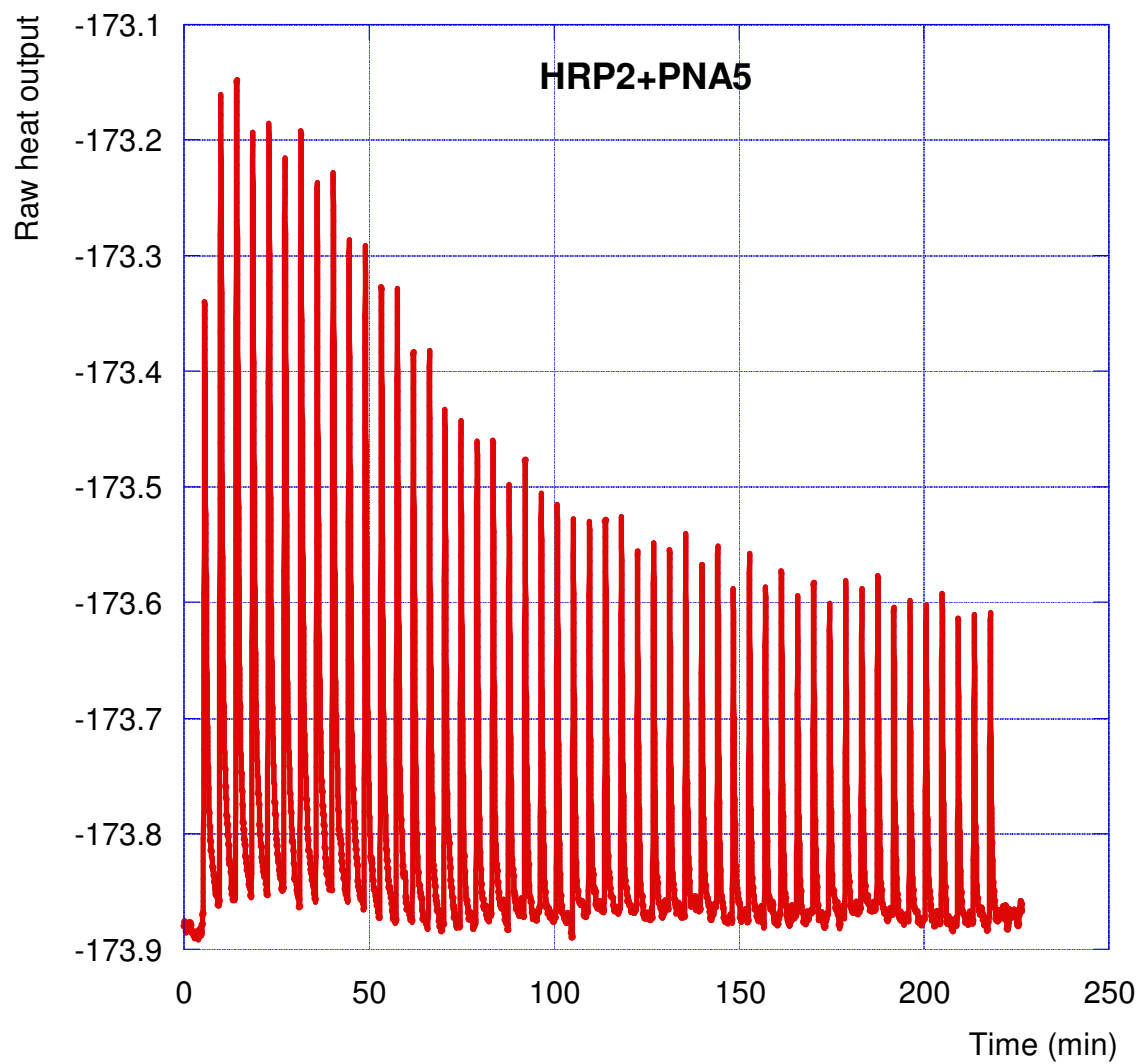


Figure S15. ITC data for binding of **PNA5** to **HRP2**.

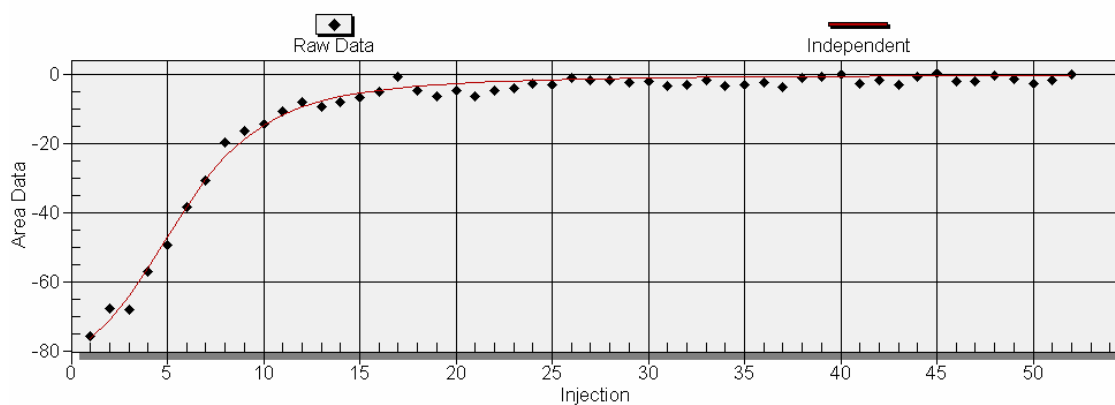
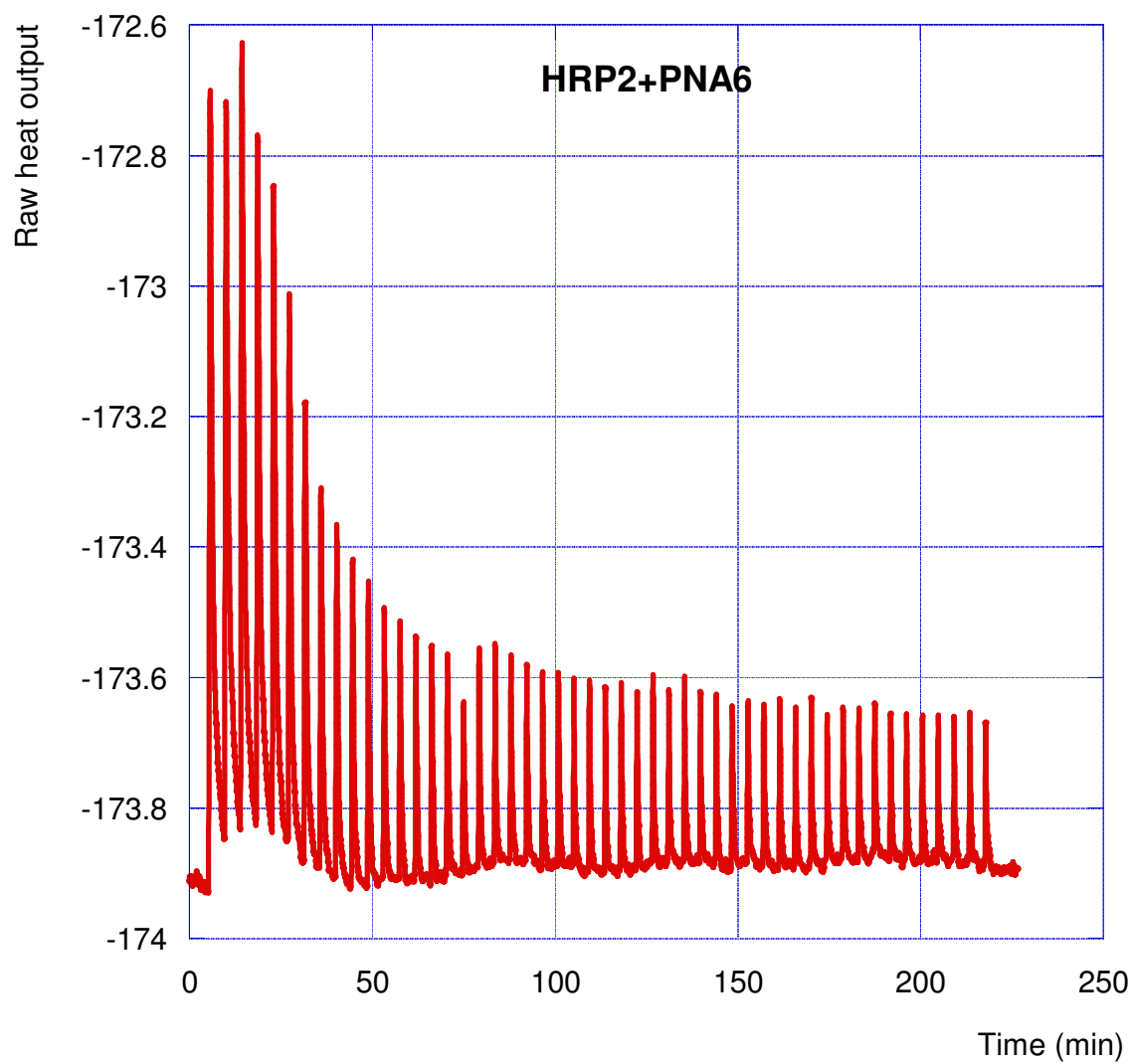


Figure S16. ITC data for binding of **PNA6** to **HRP2**.

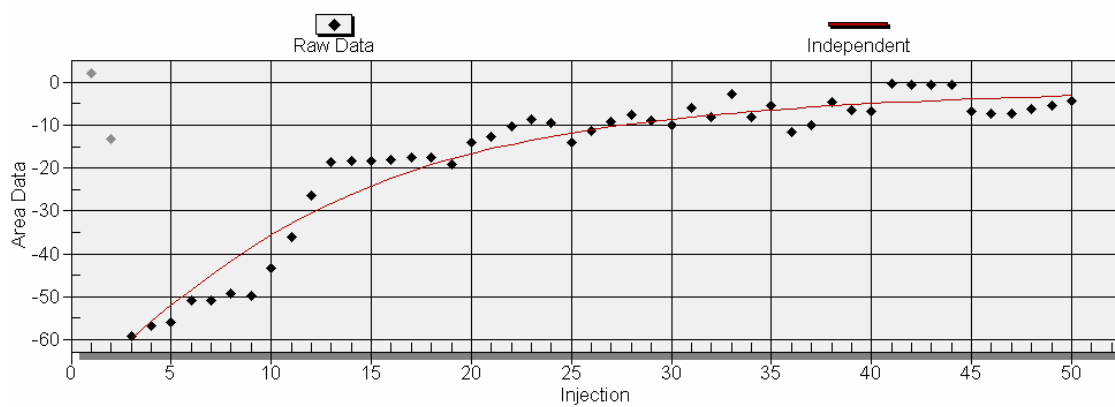
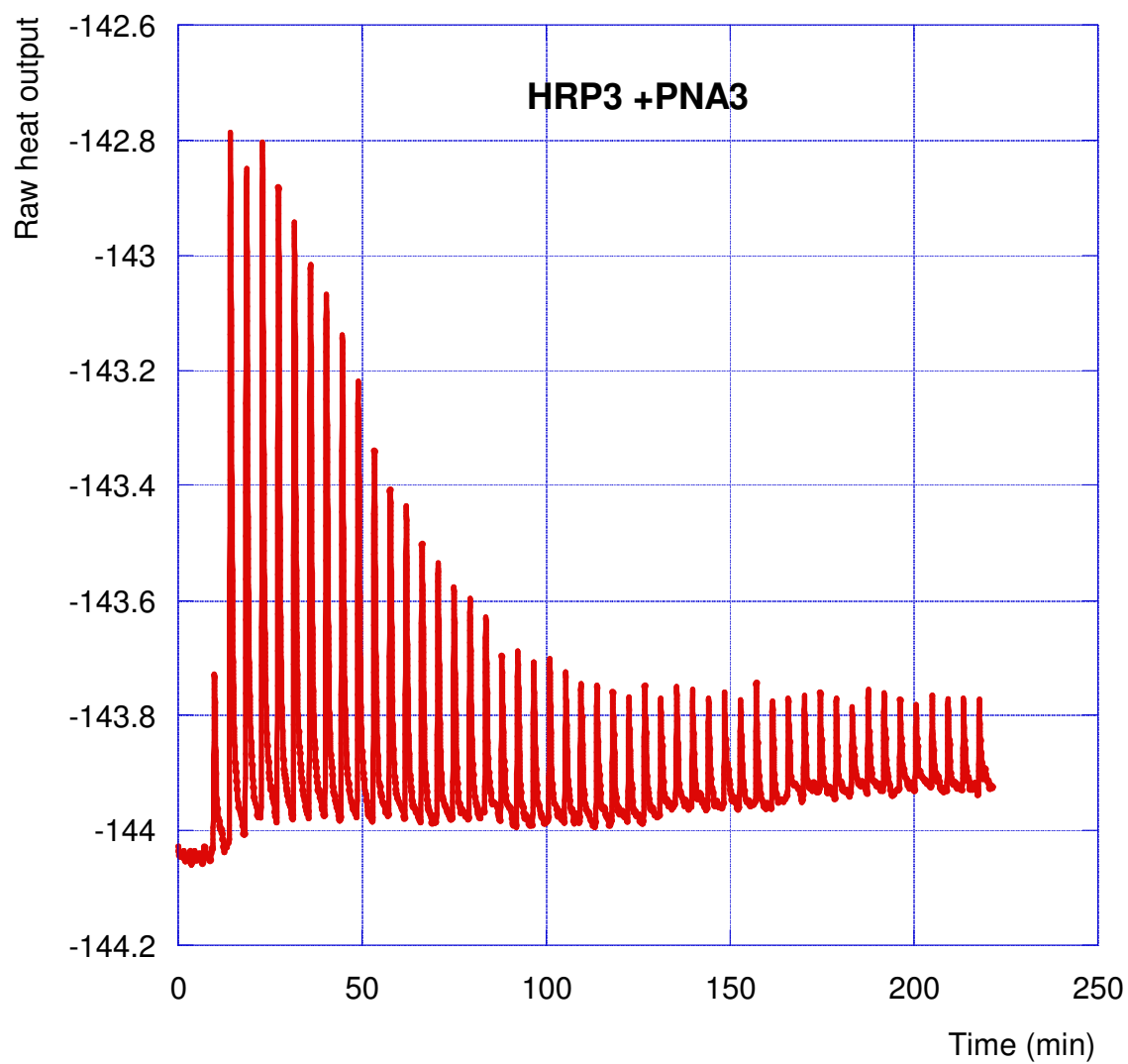


Figure S17. ITC data for binding of **PNA3** to **HRP3**.

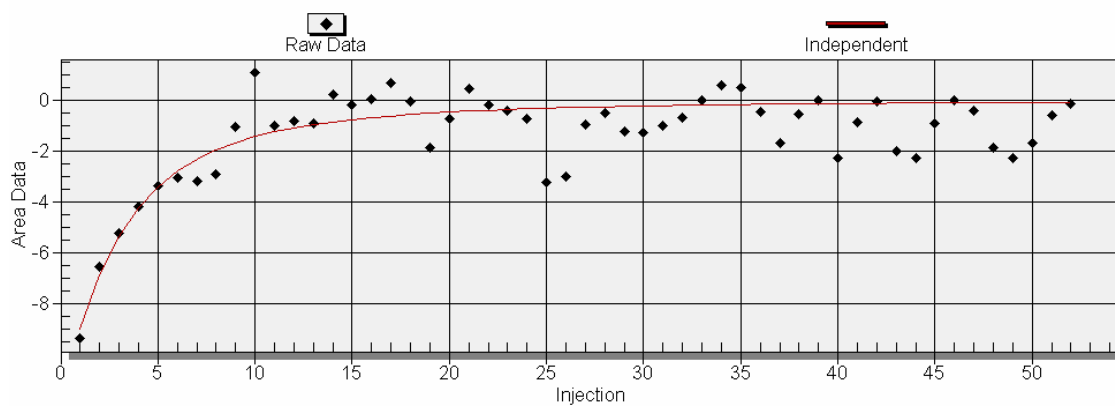
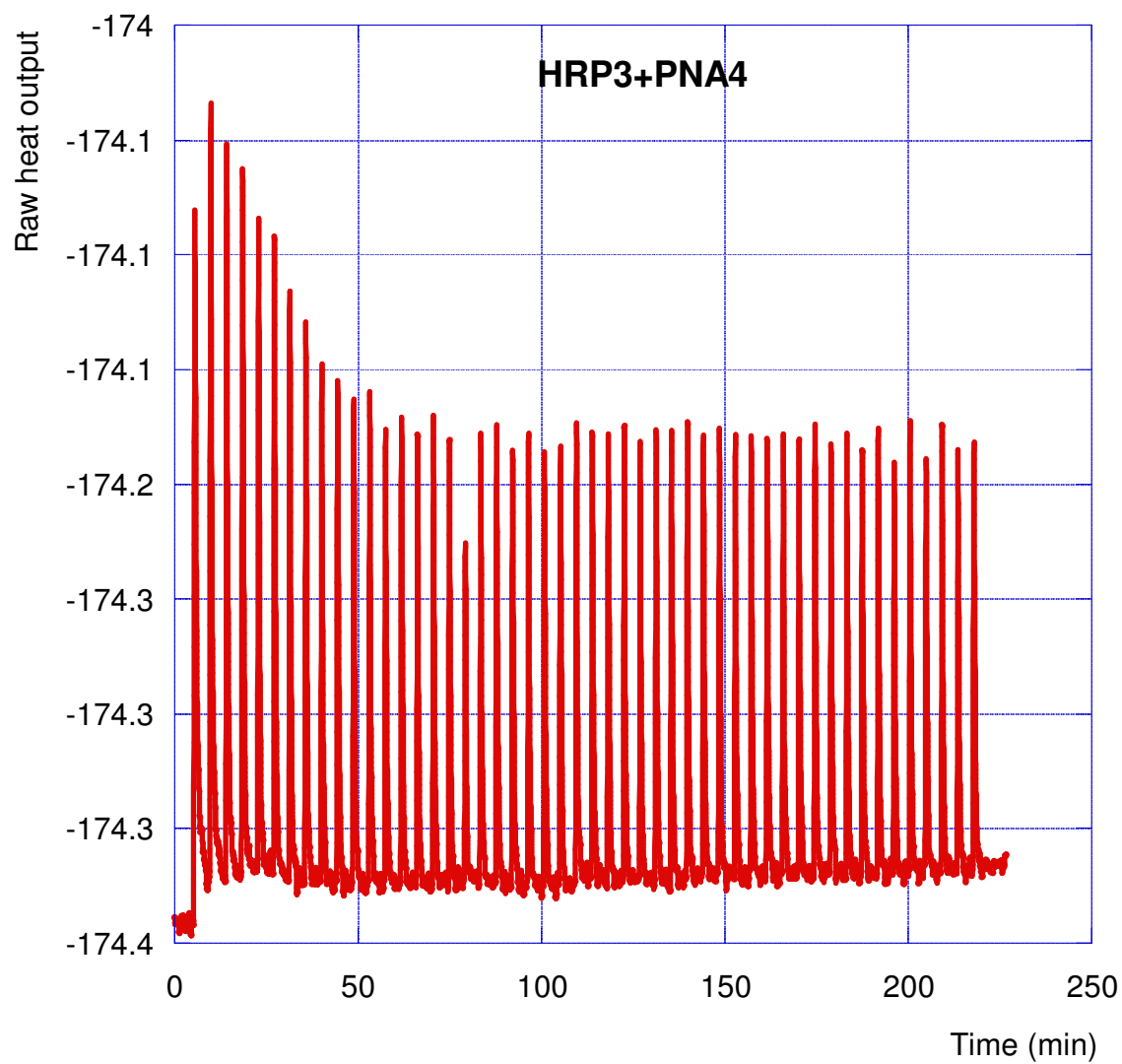


Figure S18. ITC data for binding of **PNA4** to **HRP3**.

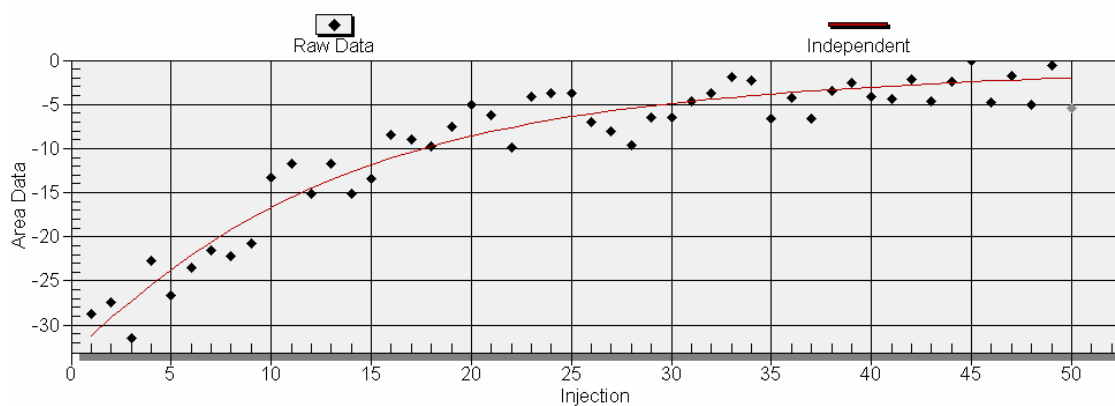
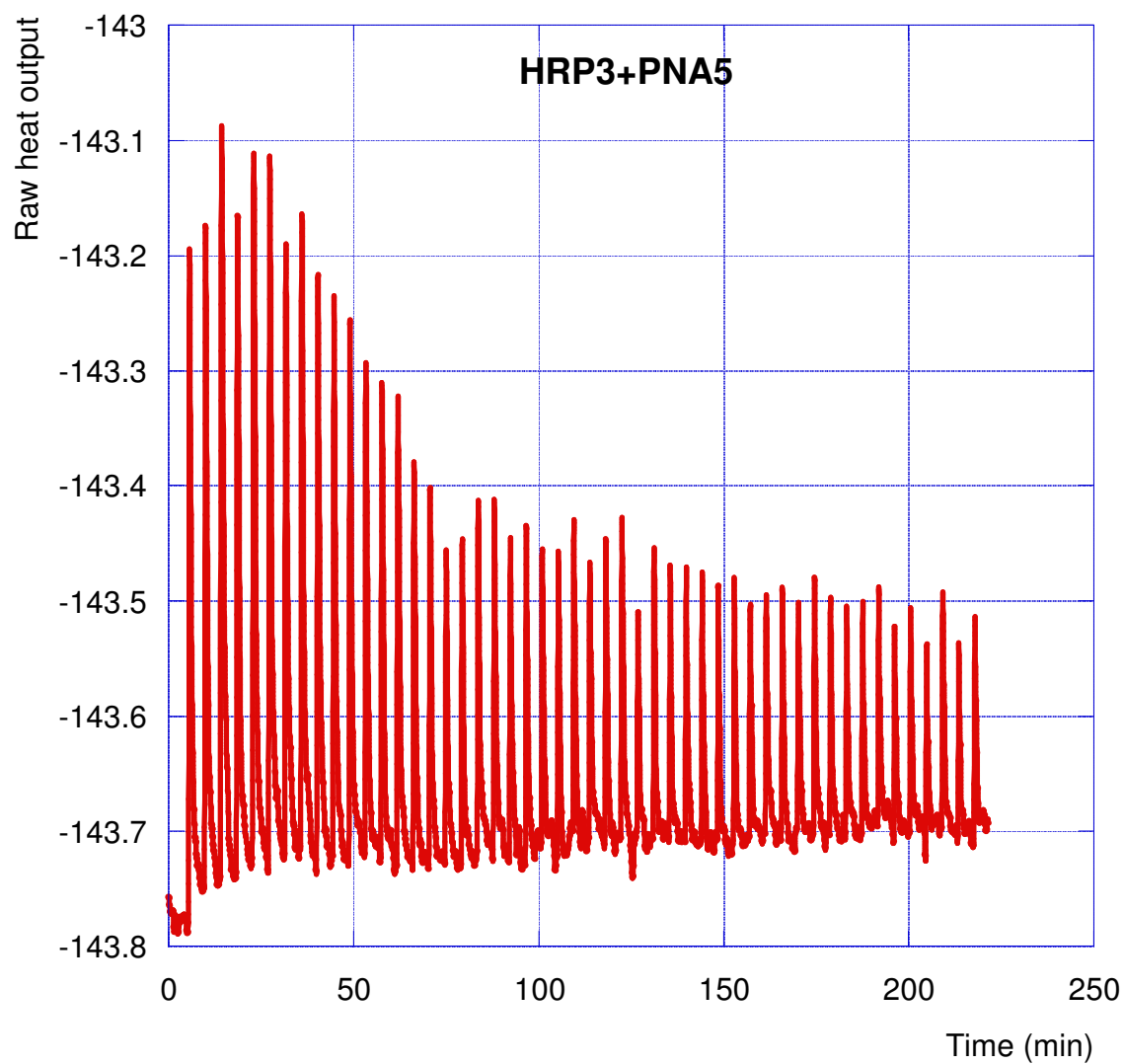


Figure S19. ITC data for binding of **PNA5** to **HRP3**.

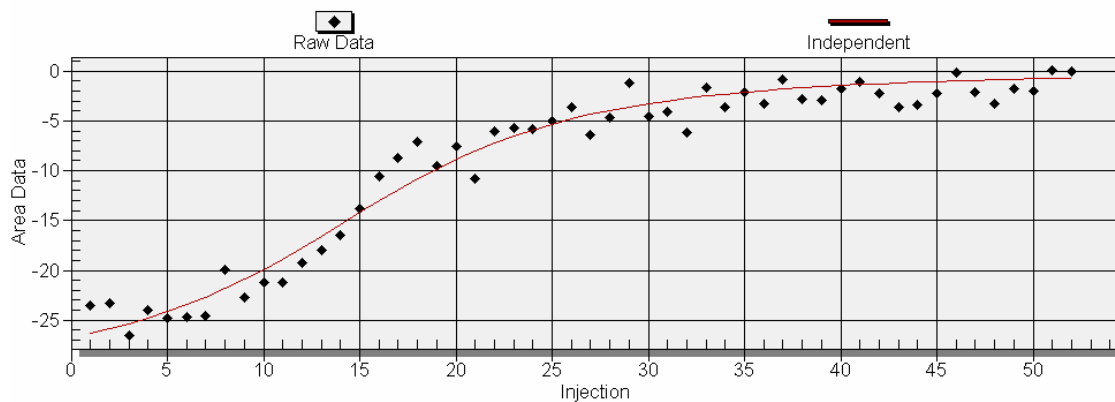
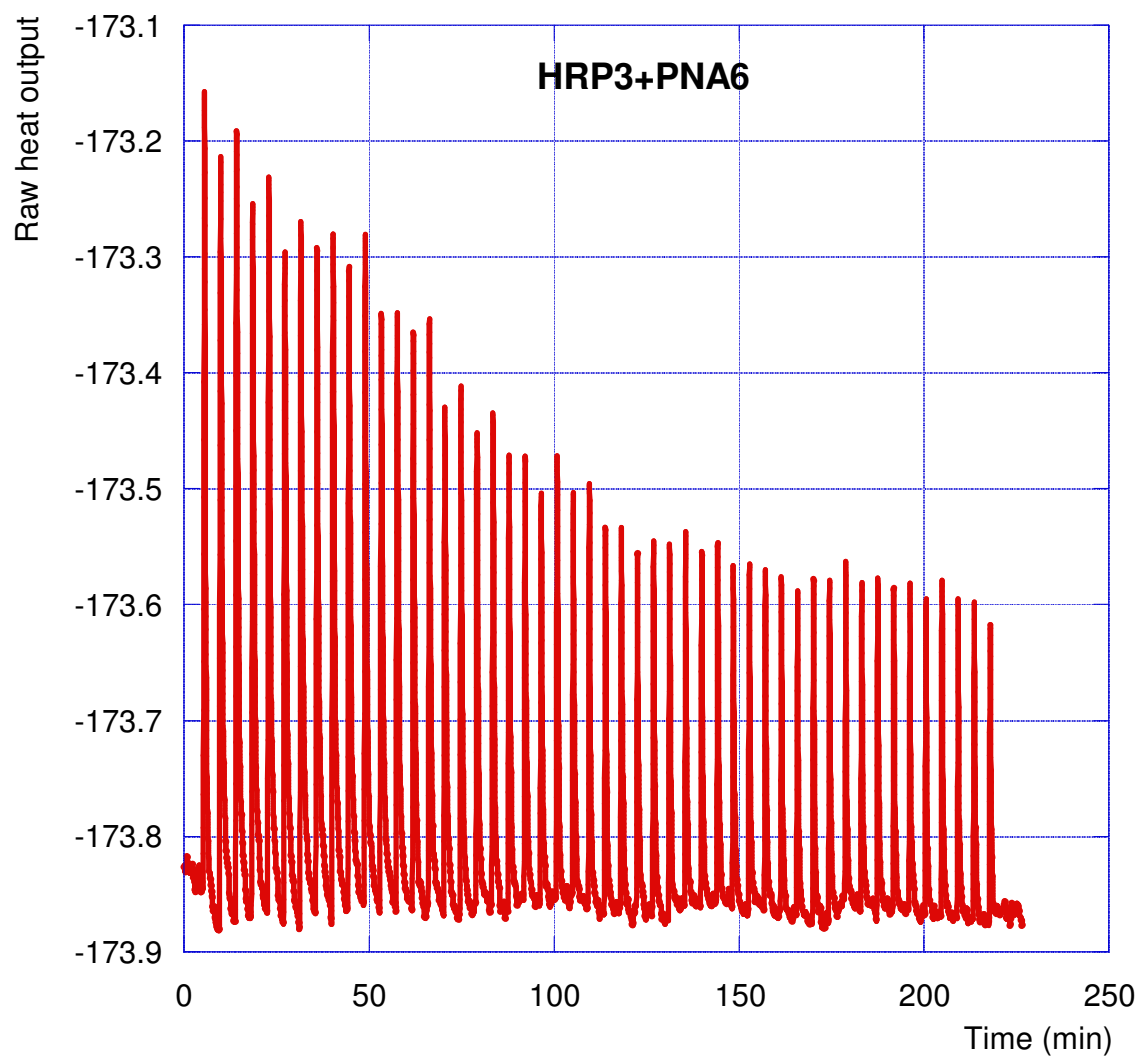


Figure S20. ITC data for binding of **PNA6** to **HRP3**.

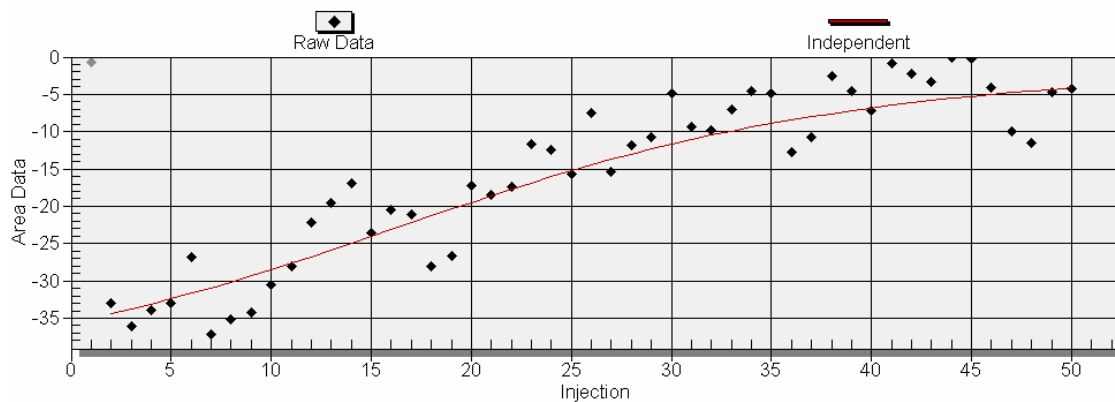
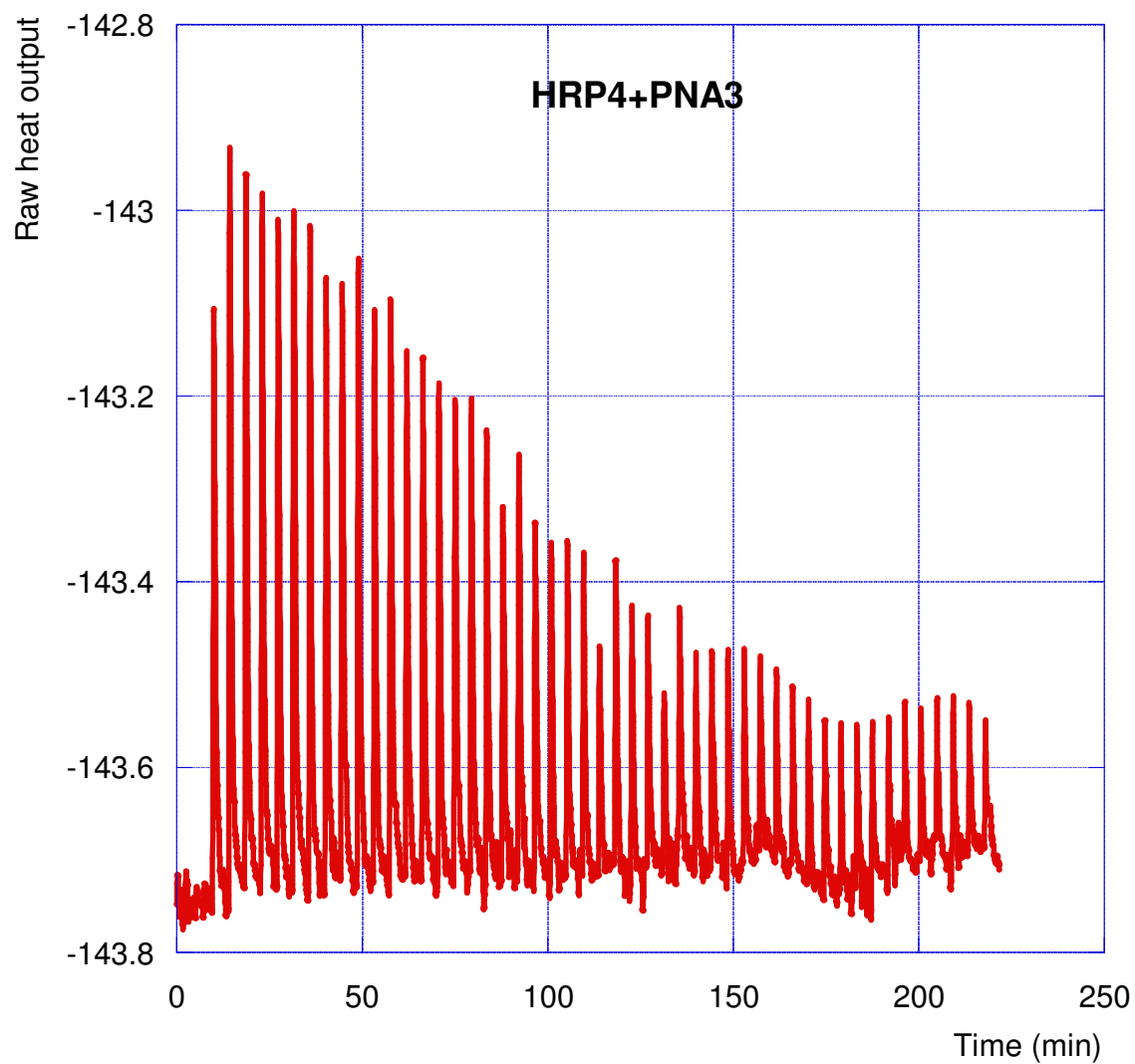


Figure S21. ITC data for binding of PNA3 to HRP4.

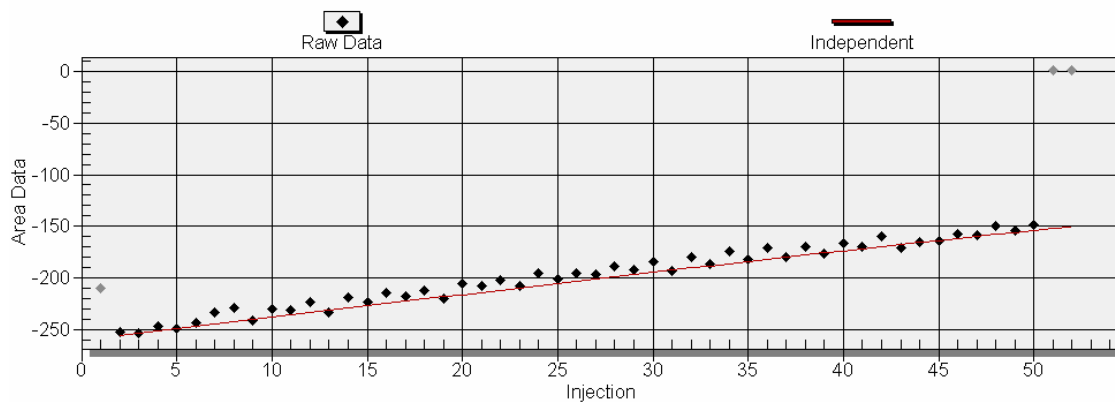
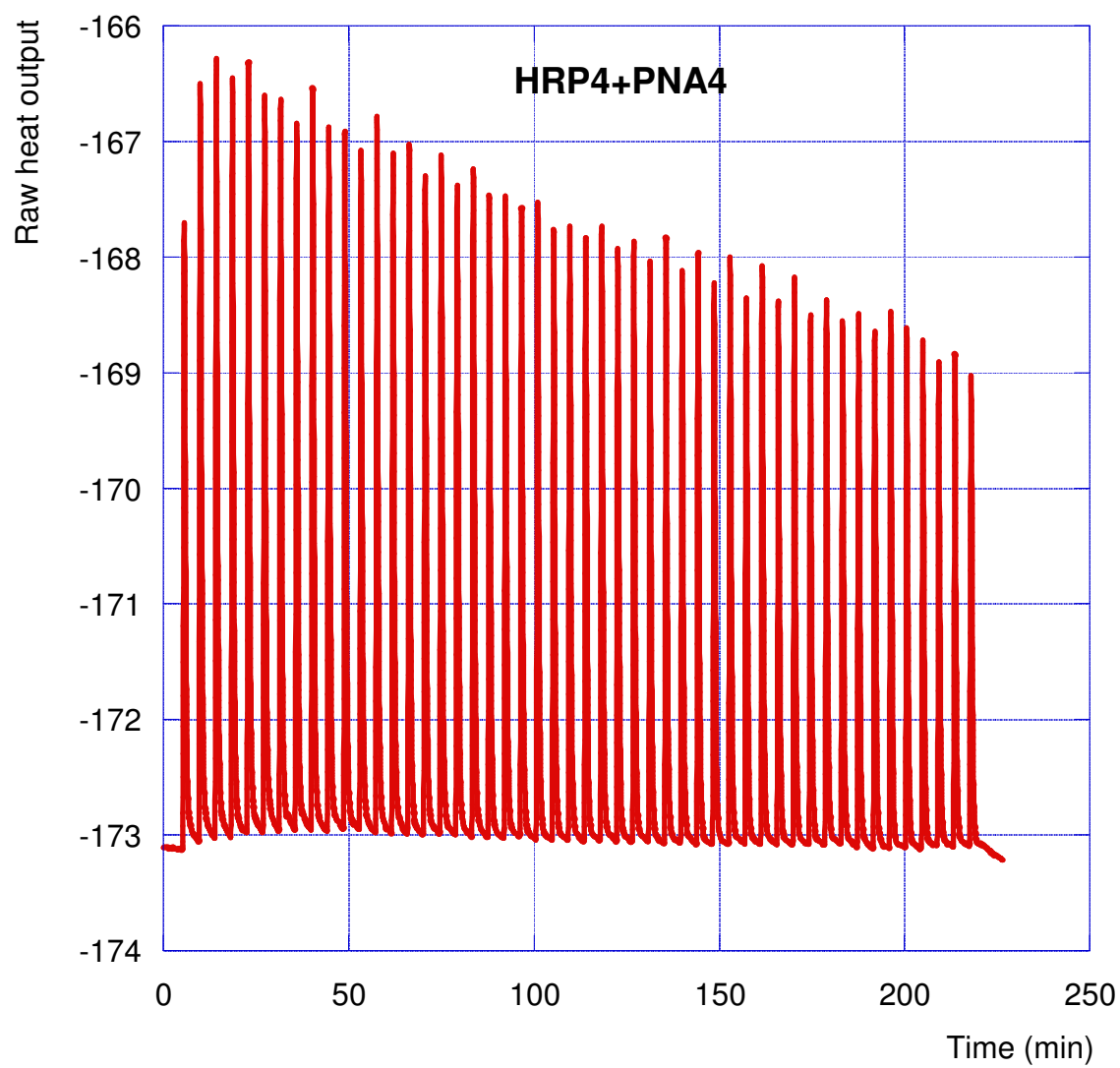


Figure S22. ITC data for binding of PNA4 to HRP4.

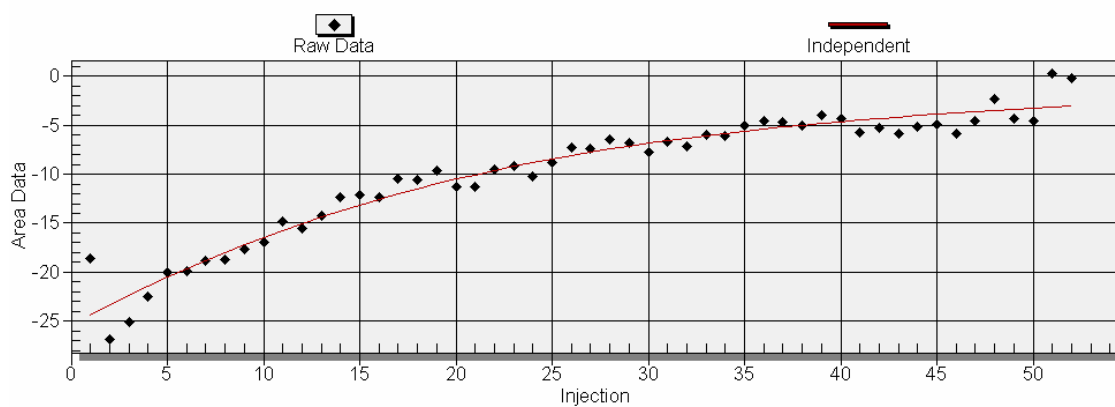
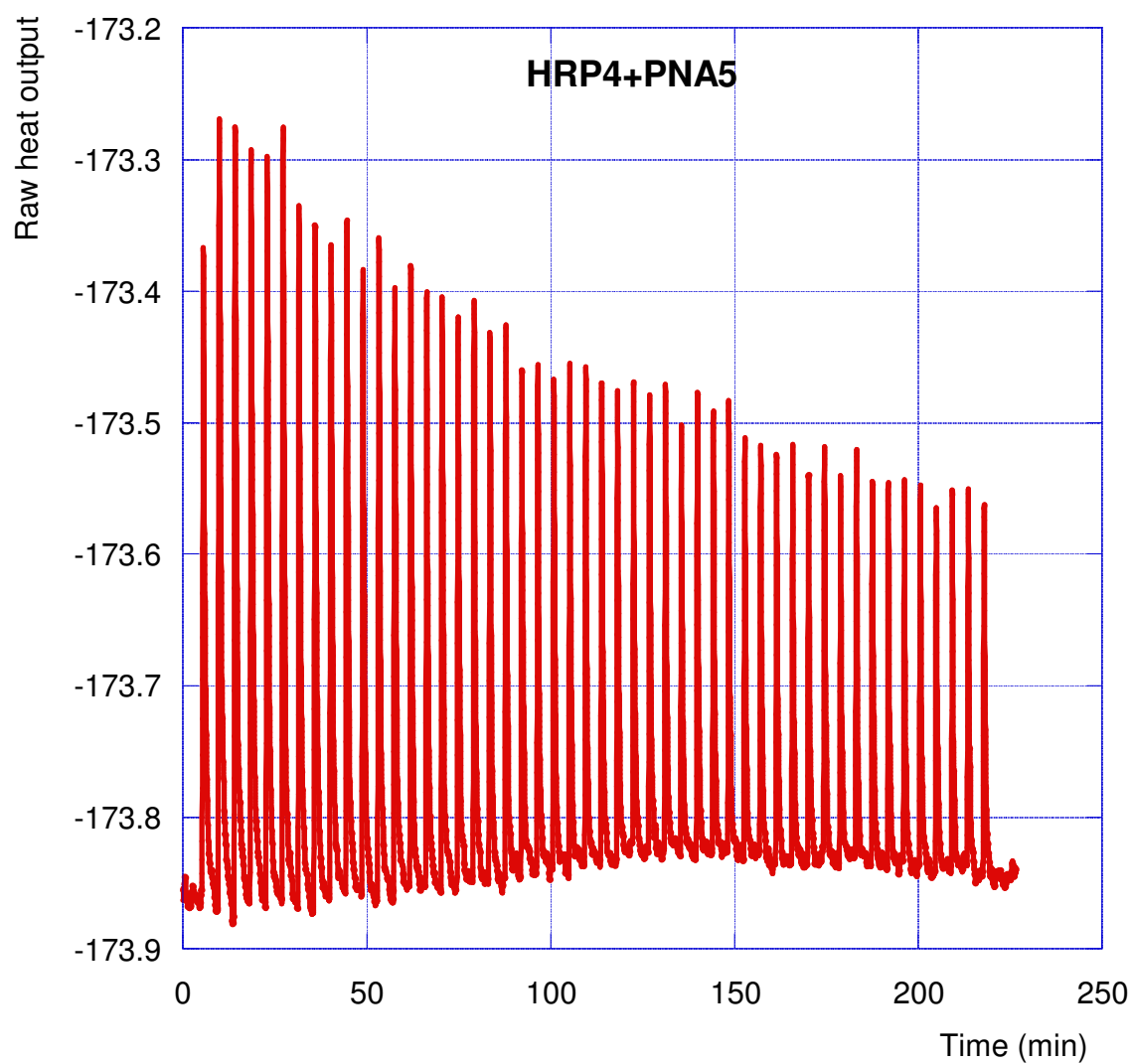


Figure S23. ITC data for binding of **PNA5** to **HRP4**.

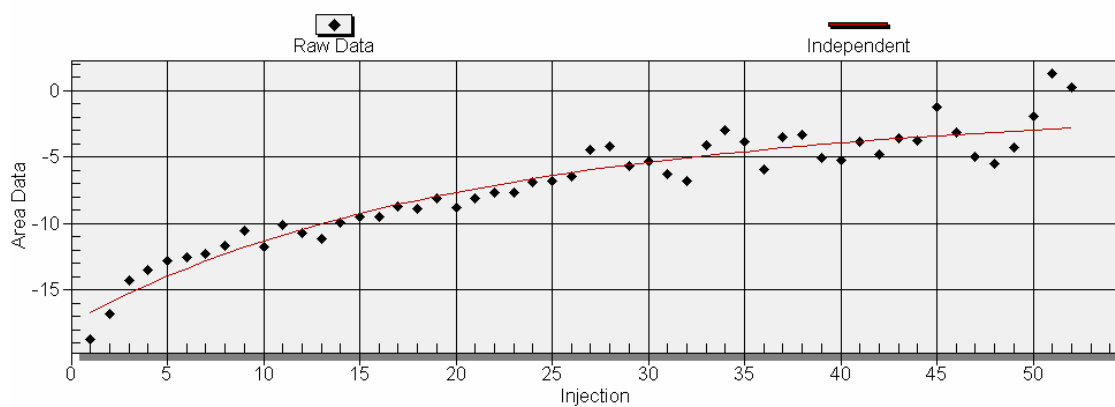
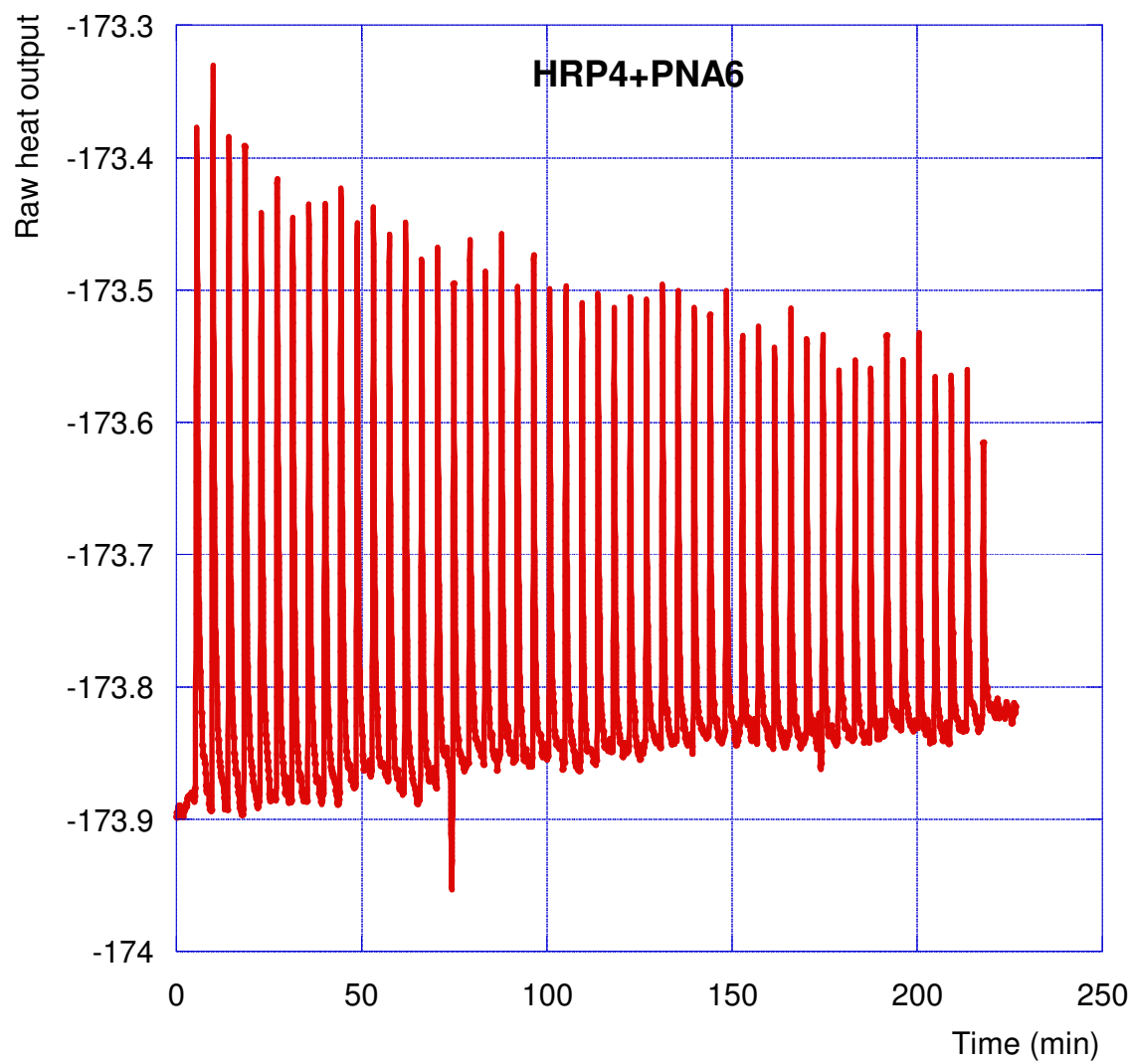


Figure S24. ITC data for binding of **PNA6** to **HRP4**.

Table S1. Experimental results of ITC titrations – analyzed data.

Sequence	K	ΔH	ΔS	ΔG	order
HRP1					
RNA	1.32E+07	-56.1	-156	-9.7	0.8
PNA1	4.34E+08	-48.7	-124	-11.8	1.3
	7.44E+08	-61.4	-165	-12.1	1.2
	1.14E+08	-54.7	-147	-11.0	1.0
	1.07E+08	-56.8	-154	-10.9	1.1
average	3.50E+08	-55.4	-147	-11.5	1.2
standard					
dev	3.04E+08	5.3	17	0.6	0.1
PNA1	reverse addition				
HRP1	9.50E+07	-83.8	-245	-10.9	0.73
	7.80E+07	-89.3	-264	-10.8	0.75
average	8.65E+07	-86.6	-254.1	-10.8	0.74
standard					
dev	1.20E+07	3.9	13.3	0.1	0.01
PNA1 pH 7	4.75E+05	-36.3	-96	-7.7	1.0
PNA2	4.51E+07	-42.5	-108	-10.4	1.3
PNA3	5.39E+07	-30.6	-67	-10.5	1.0
	5.70E+07	-32.2	-73	-10.6	1.0
	2.31E+08	-36.1	-83	-11.4	0.9
	5.33E+07	-22.9	-42	-10.5	1.3
	2.67E+07	-25.1	-50	-10.1	1.3
average	8.44E+07	-29.4	-63	-10.6	1.1
standard					
dev	8.29E+07	5.3	17	0.5	0.2
PNA4	2.63E+06	-22.7	-47	-8.8	1.3
	2.67E+06	-22.7	-47	-8.8	1.3
average	2.65E+06	-22.7	-46.7	-8.8	1.3
standard					
dev	2.83E+04	0.0	0.0	0.0	0.0
PNA5	1.54E+06	-17.4	-30	-8.4	2.8
PNA6	6.20E+06	-28.4	-64	-9.3	1.1
PNA3	5.10E+06	-82.6	-247	-9.1	1.7
HRP2					
PNA3	4.13E+05	-28.4	-70	-7.7	2.1
PNA4	6.29E+07	-24.1	-45	-10.6	1.4

	3.19E+07	-28.7	-62	-10.2	1.2
average	4.74E+07	-26.4	-53.5	-10.4	1.3
standard					
dev	2.19E+07	3.2	11.7	0.3	0.1
PNA5	3.56E+05	-25.6	-60	-7.6	1.4
PNA6	1.56E+06	-47.5	-131	-8.4	0.6
HRP3					
PNA3	3.46E+05	-56.1	-163	-7.6	0.8
	5.83E+05	-32.0	-81	-7.9	1.4
average	4.65E+05	-44.1	-122.0	-7.7	1.1
standard					
dev	1.68E+05	17.1	57.9	0.2	0.4
PNA4	4.62E+05	-97.7	-302	-7.7	0.0
PNA5	1.87E+05	-37.0	-100	-7.2	0.8
PNA6	7.98E+05	-18.6	-35	-8.0	1.3
	5.77E+05	-19.8	-40	-7.9	1.2
average	6.88E+05	-19.2	-37.8	-8.0	1.3
standard					
dev	1.56E+05	0.8	3.3	0.1	0.1
HRP4					
PNA3	2.82E+05	-25.1	-59	-7.4	2.1
	5.20E+04	-34.2	-93	-6.4	2.9
average	1.67E+05	-29.6	-76.1	-6.9	2.5
standard					
dev	1.63E+05	6.4	23.9	0.7	0.6
PNA4	1.77E+04	-373.8	-1234	-5.8	9.0
PNA5	9.45E+04	-34.4	-93	-6.8	1.4
PNA6	5.29E+04	-85.7	-266	-6.4	0.5